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# SOIL SCIENCE

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SOIL BIOLOGY

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# ULTIMATE ANALYSIS OF THE MINERAL CONSTITUENTS OF A HAGERSTOWN SILTY CLAY LOAM SOIL AND OCCURRENCE IN PLANTS OF SOME OF THE ELEMENTS FOUND<sup>1</sup>

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## INTRODUCTORY

The soil for which analysis is here reported is being used in a 20-year cooperative experiment between the Departments of Horticulture and of Experimental Agricultural Chemistry. This experiment is being conducted in steel rims, forty-two in number, to determine the effect of fertilizers on the yield, growth, and other physiological functions of the apple tree. It is the result of inconclusive chemical studies conducted during 1917-19 on the soils and plants of the experimental orchards throughout the state. The variables have been eliminated in the present investigation as nearly as possible by using a uniform soil obtained by thorough and complete mixing of the sub-, subsurface, and surface-soil and also by means of a uniform stock. The soil with which these rims were filled was taken from a strip of virgin land adjacent to the experimental orchard, which lies about a mile and a half to the northeast of the well known general fertilizer plats, at the junction of the Stonehenge and Trenton formations, and is classed by Mooney, Shaw et al. (55), as a clay loam of the Hagerstown series. However, Shaw recognizes considerable variations in the texture and other physical qualities of these soils, e.g. he distinguished as present in the general fertilizer plats the following sub-classes: silty clay loam, heavy loam, clay loam and clay. The mechanical analyses (cf. table 1) of the soil in this experiment would indicate that it is a mixture of the Hagerstown silt loam and clay loam. This soil is probably formed by the disintegration of limestone rocks and represents that portion of the soil mass adjacent to the parent rock.

In an intensive experiment of this character it was regarded as fundamental to have as complete a knowledge not only of the total constituents of the soil

<sup>1</sup> Contribution No. 1 from the laboratories of the Department of Agricultural Chemistry of the Pennsylvania State College Agricultural Experiment Station.

<sup>2</sup> The mineralogical section of this paper (cf. p. 18) is based on the micro-petrographical examination made by Professor A. P. Honess. The author wishes to take this opportunity to express his indebtedness and gratitude for the guidance given him by the late Dr. William Frear with whom the author has been associated for nearly 10 years and under whose general direction the chemical phase of this project was initiated.



but, also of the total amount of each element present, be obtained by making a complete examination following the fusion method for rock analyses and adopting quantitative procedures of approved validity.

Robinson (62, p. 12-13) has reported the occurrence of a number of the rarer elements in a Hagerstown loam soil taken one mile northwest of Conshohocken, Pa., which is not far from metamorphic rocks. Fragments of mica schist (1.8 per cent of the stony fragments) occur in the surface soil. This showing is quite different from the conditions at State College, where the soil has been formed in place by weathering of the limestone belonging to the lower Silurian formations. The existence of these elements in this soil is conceivably susceptible of several explanations. If strictly residual then the origin would be the underlying limestone, but it is very questionable if these rarer elements would be found in the limestone underlying the soil. This point will be investigated later. As Merrill (54, p. 4-8) points out, the agencies which have been instrumental in the formation of soils have been so complex that many of our soils are but secondary rocks in a state of loose consolidation, and many of the accumulations classed as residual have been derived by disintegration in situ of alluvial materials which have been brought down years ago and deposited in shallow seas. Moreover, because of the transporting power of water, wind, or moving ice, few residual deposits have retained their virgin purity, but have become more or less contaminated with materials from near or distant sources.

Dr. E. S. Moore, Dean of the School of Mines of the Pennsylvania State College is of the opinion that these rarer elements have, probably, been derived "from the extensive pre-Cambrian area, known as the Appalachia, which existed to the east and northeast of this region at the time these Palaeozoic rocks were being laid down."

#### THE ISOLATION OF THE RARER ELEMENTS IN THIS RESIDUAL SOIL

Clarke (11, p. 15-22), Merrill (54, p. 312), Washington (75), and Hillebrand (42, p. 25-30) have been consulted in obtaining the following data on the soil used in this study:

*Aluminum:* Found chiefly in silicates such as the feldspars and the micas. It occurs in this soil in the mixed feldspars,  $R'AlSiO_4$ , in which  $R'$  may be Ca, Mg, Na or K; mica,  $H_2(KNa)Al_4(SiO_4)_2$ ; tourmaline,  $R'_3Al_3B_3(OH)_3Si_4O_{12}$ , in which  $R'$  may be Fe, Mg or alkali metals; and chlorite,  $H_2(Mg,Fe)_2Al_2(SiO_4)_2$ .

*Barium:* Widely distributed in small quantities throughout igneous rocks. Professor Honess has found barite in sediments near Bellefonte, Pa., and has called the attention of the writer to the statement by Dana (19) that it has been proven to exist in amounts varying from 0.45 per cent to 2.20 per cent in orthoclase and some acid plagioclases, which are probably the original sources of barium, from which it is dissolved. It occurs also as carbonate and witherite. Failyer (27) reports that barium is present in most soils throughout the United States.

*Boron:* An essential constituent of several silicates. It is present in this soil in tourmaline, which has been found by Doctor Moore in the Tuscarora sandstones and quartzites.

*Cesium:* A rare metal—the rarest of the alkalis. Often found in lepidolite,  $(Li,K)_2(FOH)_2Al_2SiO_5$ ; Vernadski (69, 70) found spectroscopic traces in the feldspars and micas, which are the probable sources of the element in this soil.

*Calcium:* An essential constituent of many rock-forming minerals such as the amphiboles and the pyroxenes. It occurs as dolomite,  $CaMg(CO_3)_2$ , and amphibole,  $Ca(MgFe)_2(SiO_4)_2$ . Sericite and the mixed feldspars found in this soil contain small amounts of calcium.

*Chromium:* Very widely diffused in the form of chromite,  $(Fe,Cr)[(Cr,Fe)O_2]_2$ , and especially in ferro-magnesian rocks. Its source in this soil is probably chlorite.

*Fluorine:* Fluorine is found in a number of silicates such as tourmaline and the micas, which are its probable source in this soil.

*Iron:* Found in practically all rocks. Occurs as limonite,  $H_2Fe_2O_4(H_2O)_4$ ; ilmenite  $FeTiO_3$ ; magnetite,  $Fe_3O_4$ ; and also chlorite and amphibole in this soil.

*Lithium:* Traces are found in nearly all igneous rocks. The most important lithia minerals are lepidolite,  $(Li,K)_2(F,OH)_2Al_2Si_4O_{10}$ ; spodumene  $LiAl(SiO_3)_2$ ; and the lithia tourmalines and alkali feldspars. Washington (75, p. 20) states that it frequently occurs in rocks high in sodium.

*Magnesium:* Magnesium occurs in this soil as amphibole, dolomite and chlorite. The micas also contain magnesium.

*Manganese:* Found in most rocks and soils in small amounts in the ferro-magnesian minerals. Through alteration it appears sometimes on the surfaces of limestones and sandstones. Its probable source in this soil is chlorite.

*Molybdenum:* Hillebrand (42, p. 185) has found it in minute quantities in siliceous rocks. It has also been found in granite in the form of molybdenite,  $MoS_2$ , but its source in this soil has not been determined.

*Phosphorus:* Found in nearly all igneous rocks in the mineral apatite. Its source in this soil has not been determined unless it occurs in the limonite (cf. p. 11).

*Potassium and sodium:* These metals are present in this soil in the mixed feldspars and micas.

*Rubidium:* The source of this metal in this soil has not been determined. It is found in lepidolite,  $(Li,K)_2(F,OH)_2Al_2Si_4O_{10}$ , and in some mineral springs. It is reported as being present in the waters of the Caspian Sea.

*Strontium:* This is a common ingredient of igneous rocks in small amounts. The most important minerals are celestite,  $SrSO_4$ , and strontianite,  $SrCO_3$ . Lesley (47) reports the absence of celestite in the beds of this limestone formation. Professor Honess, however, has called to the author's attention the fact that Dana (20) states that it occurs in the limestone regions of Central Pennsylvania, at Bells Mills, Blair County. It has recently been found by Mr. A. H. Dewey of the School of Mines of this Institution at Williamsburg, Pa.

*Sulfur:* The source of sulfur in this soil has not been determined. It certainly exists as sulfates and in organic combination. No sulfide minerals were found.

*Titanium:* Titanium is almost invariably present in igneous rocks and the sedimentary material derived from them. It occurs in this soil as ilmenite,  $FeTiO_3$ , and rutile,  $Ti_2TiO_4$ .

*Vanadium:* This is diffused with titanium through all primitive granite rocks, has been found in rutile and bauxite, and is reported as occurring very commonly in small amounts in residual limestone deposits, which is probably the source of the vanadium found in this soil. Phillips (58) suggests that vanadium in sedimentary rocks may be derived from the decay of mineral organisms—the acidians.

*Zirconium:* This is allied to titanium and rather widely diffused in the igneous rocks and has been found by Doctor Moore in the Tuscarora sandstones and quartzites. Hillebrand (42, p. 25-30) and Robinson (62, p. 3) state that it occurs in largest amounts in soils high in silica and sodium.

The present investigation is of special interest because of the isolation by quantitative methods of known validity and confirmatory qualitative tests of the rarer elements vanadium, chromium, molybdenum, zirconium, titanium, rubidium, caesium, lithium and also of the elements barium and strontium, concerning the presence of which in this soil series much doubt has existed.

#### THE DISTRIBUTION OF THE ELEMENTS PRESENT IN THIS SOIL MECHANICAL ANALYSIS

The mechanical analysis was carried out by the method described by the Bureau of Soils (8, p. 7-24) on samples prepared according to the methods

prescribed by the Association of Official Agricultural Chemists (2) dried at 110°C. Duplicate determinations were made and are shown in table 1.

It is of interest to note the difference in mechanical composition of this soil and that of the grass lands of the general fertilizer plats as given by Frear and White (30), in table 2.

The soil used in the apple rim experiment is, therefore, of a much more clayey texture than the major portion of the land of the college farm, though the relatively larger amount of clay present is not sufficient to interfere with the ease of tillage, excellence of drainage and formation of clods on drying, which are characteristic of this soil type.

TABLE 1

GRADE	SURFACE SOIL TO 9.5 IN.		SUBSURFACE SOIL 9.5-18.5 IN.		SUBSOIL 18.5-42 IN.	
	(a)	(b)	(a)	(b)	(a)	(b)
	per cent	per cent	per cent	per cent	per cent	per cent
Coarse sand, 1.0-0.5 mm.....	7.30	7.52	5.45	5.43	4.43	4.45
Medium sand, 0.5-0.25 mm.....	4.82	5.20	3.90	3.74	3.51	3.40
Fine sand, 0.25-0.1 mm.....	3.40	3.40	3.07	3.00	3.05	3.01
Very fine sand, 0.1-0.05 mm.....	12.80	12.51	14.00	13.98	12.22	12.50
Silt, 0.05-0.005 mm.....	50.30	50.52	45.43	46.00	46.45	47.44
Clay, 0.005 mm. or less.....	15.78	15.67	23.29	22.76	25.57	24.33
Loss on ignition.....	5.30	5.30	5.00	5.00	5.50	5.50
Total.....	99.70	100.12	100.14	99.91	100.73	100.63

TABLE 2

GRADE	SURFACE SOIL TO 7 IN.	SUBSURFACE, 7-14 IN.
	per cent	per cent
Very fine sand.....	6.44	6.78
Silt.....	75.05	67.83
Clay.....	9.45	17.98

Samples were taken from each wheelbarrow load as the rims were being filled. These were placed in cans and stored as "surface-", "subsurface-" and "sub-" soil. The samples from each of these three layers were treated as follows: The whole soil sample, about 270 kgm., was placed upon heavy paper, thoroughly mixed by turning over with a hand scoop, and divided into four segments in the usual way. The process of mixing and quartering was continued until the quantity of soil was reduced to about 2 kgm.

The three samples resulting were air-dried, washed and sieved as prescribed by the method of the Association of Official Agricultural Chemists (2). About 50 gms. of each sample, was finely ground in an agate mortar until all passed through a two hundred mesh silk bolting cloth.

## THE CHEMICAL ANALYSIS

The complete chemical analysis of the soil, in general as outlined by Hillebrand (42), is given in table 3.

The analysis of the various soil fractions has not been undertaken. Failyer, Smith and Wade (28) have shown that as a general rule the smaller particles of soils are richer in potassium, calcium, magnesium and phosphorus than

TABLE 3

CONSTITUENT	SURFACE SOIL TO 9.5 IN.		SUBSURFACE SOIL 9.5-18.5 IN.		SUBSOIL 18.5-42 IN.	
	(a)	(b)	(a)	(b)	(a)	(b)
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
SiO <sub>2</sub> .....	72.320	72.340	67.720	67.620	63.230	63.000
Al <sub>2</sub> O <sub>3</sub> .....	10.192	10.322	13.503	13.667	15.994	15.974
Fe <sub>2</sub> O <sub>3</sub> .....	3.710	3.670	5.580	5.580	6.840	6.840
FeO.....	0.540	0.550	0.350	0.350	0.207	0.207
V <sub>2</sub> O <sub>5</sub> .....	0.036	0.036	N E	N E	0.090	0.080
Cr <sub>2</sub> O <sub>3</sub> .....	0.002	0.002	N E	N E	0.002	0.002
MoO <sub>3</sub> .....	P*	P	N E	N E	P	P
TiO <sub>2</sub> .....	0.340	0.320	0.480	0.460	0.440	0.480
P <sub>2</sub> O <sub>5</sub> .....	0.102	0.095	0.102	0.098	0.077	0.078
ZrO <sub>2</sub> .....	0.037	(0.037)†	0.045	(0.045)	0.050	(0.050)
MnO.....	0.240	0.230	0.250	0.260	0.230	0.260
CaO.....	0.660	0.700	0.650	0.660	0.900	0.910
MgO.....	0.650	0.680	0.980	1.050	1.370	1.350
BaO.....	0.009	(0.009)	0.019	0.020	0.019	0.019
SrO.....	0.016	0.010	0.022	0.018	0.033	0.028
Rb.....	0.001	0.001	N E	N E	0.001	0.001
K <sub>2</sub> O.....	3.960	3.890	4.220	4.180	4.420	4.400
Cs.....	N F	N F	N E	N E	P	P
Na <sub>2</sub> O.....	2.280	2.240	1.280	1.300	1.350	1.280
Li.....	Trace	Trace	Trace	Trace	Trace	Trace
Cl.....	Trace	Trace	Trace	Trace	Trace	Trace
S.....	0.062	(0.062)	0.051	(0.051)	0.062	(0.062)
SO <sub>3</sub> .....	0.017	0.017	0.020	0.020	0.017	0.017
Loss on ignition.....	5.300	(5.300)†	5.000	(5.000)	5.500	(5.500)
Total.....	100.474	100.511	100.272	100.379	100.832	100.538
Less oxygen equivalent of S.....	100.448	100.485	100.250	100.357	100.806	100.512

\* P = present; N E = not examined; N F = not found.

† Parentheses indicate that duplicate determinations were not made.

the coarser particles and the concentration becomes greater the greater the weathering, and that the larger mechanical fractions contain these elements in forms which by protracted weathering will become more soluble and will ultimately be concentrated in the finer compounds.

The mineralogical examination of this soil (cf. p. 10) confirms these conclusions.

## NOTES ON THE CHEMICAL ANALYSIS

1. Before mixing with sodium carbonate prior to the fusion, the charge was ignited to destroy organic matter.

2. The silica not obtained by the fusion with HCl, after three evaporations and intervening filtrations (41), was separated from the mixed oxides, of alumina, etc., by fusion with potassium bisulfate and added to the weight of the silica already obtained. The weight of silica was corrected for impurities by evaporation in HF and H<sub>2</sub>SO<sub>4</sub>.

3. The precipitation of iron and aluminum was made with ammonia by Blum's procedure (6). The author found no manganese, alkaline earth metals or magnesium in this precipitate.

4. Manganese was determined by the ammonium sulfide method. The amount of manganese that escaped precipitation was negligible, for none was found by the colorimetric method in the magnesium pyrophosphate precipitate in which, if any was present, it would occur.

5. The results for ferrous iron are to be regarded as approximate. The various devices used to prevent oxidation of the FeO to Fe<sub>2</sub>O<sub>3</sub> are fully discussed by Hillebrand (42, p. 189-192) and Washington (75, p. 122-126). The presence of organic matter, moreover, might reduce some of the ferric iron during the operations. Cooke's method (15) simplified by Barneby (3) was adopted.

6. Nickel, cobalt, copper, zinc, mercury, silver, tin, lead and arsenic were not found.

7. Calcium and strontium were precipitated as oxalates and separated, after conversion into the nitrates, by the ether-alcohol method (32). The strontium was weighed as the sulfate, which was examined by the spectroscope.

The orange  $\alpha$  line of wave length 6060 and the blue  $\delta$  line of wave length 4607.5 identified Sr. The band spectrum of calcium with characteristic line of wave length 4226.9 was also observed. This examination confirms the presence of strontium in this soil, though complete separation from calcium was not effected. It is remarkable that, though barium was absolutely identified, no barium lines were observed in this separate, showing complete separation from this element.

8. Barium together with zirconium, total sulfur and rare earths were determined by fusion with sodium carbonate and a little potassium nitrate on a separate 2-gm. charge of the soil. The zirconium and rare earths were separated from barium by means of dilute warm sulfuric acid. After evaporating the residue remaining undissolved with hydrofluoric acid and sulfuric acid, it was freed from calcium and strontium by repeated fusions with sodium carbonate, solution of the melts in water and reprecipitation with sulfuric acid. The barium sulfate obtained in this manner was dissolved in concentrated sulfuric acid and reprecipitated by water to remove the last traces of calcium. The barium sulfate thus obtained was ignited, weighed and examined by the spectroscope.

The green barium line of wave length 5535.7 was used for identification. Separation from calcium and strontium was completed, as none of the characteristic lines of these elements were present.

9. The method which Robinson (62, p. 7; 64), used to estimate the amounts of rubidium and caesium was adopted. Ten-gram portions of soil were used. The method removes a large part of the potassium and sodium chlorides present by fractional precipitation with platinic chloride and then by strong hydrochloric acid. The platinum was removed from the double platinic chlorides Cs<sub>2</sub>PtCl<sub>6</sub> and Rb<sub>2</sub>PtCl<sub>6</sub> by Horsch's method (44). After filtering and evaporation, the weights of the mixed chlorides from the surface and subsoil were 0.8 and 0.2 mgm. respectively. After treatment with hydrochloric acid, it was filtered into small vials and examined by means of the spectroscope, using the flame spectrum, according to the method of Gooch and Phinney (37).

The blue rubidium lines of wave length 4215.6 and 4202 were clearly defined in both the case of the surface and subsoil. In the case of the subsoil, but not of the surface soil, a doublet of wave length 4593.3 and 4555.4 was plainly discernible.

10. The examination for lithium was made on the solution obtained after removal of the potassium as potassium platonic chloride from the mixed chlorides of potassium and sodium. The platinum was removed by Horsch's method. The lithium lines of wave length 6708.2 and 5104 were observed.

An attempt was made to effect a separation of the lithium and sodium by Gooch's method (36), but the amounts of lithium chloride obtained were too small to obtain pure.

11. Calcium was always found in the magnesium pyrophosphate and was removed by treatment of the latter with dilute sulfuric acid and alcohol. The calcium sulfate obtained was filtered, weighed, and suitable corrections made in the figures for calcium and magnesium oxides.

12. The zirconium, rare earths, and total sulfur were determined by fusing 2-gm. portions of the soil powder (see note 8).

The zirconium pyrophosphate obtained by ignition of the phosphate was freed from any titanium that might possibly be present by fusion with sodium carbonate, leaching with water, fusion of the residue with potassium pyrophosphate, solution of the melt in 20 per cent sulfuric acid [Recolardot and Reglade (61)] to prevent contamination with titanium, iron, and chromium with the addition of hydrogen peroxide and reprecipitating with sodium hydrogen phosphate. The precipitate was ignited and weighed as the pyrophosphate.

Certainty as to its identification was again established by fusing with sodium carbonate, leaching, igniting the residue, fusing with potassium pyrophosphate and reprecipitating with ammonia, filtering, igniting and weighing as  $ZrO_2$ . This was then dissolved in concentrated sulfuric acid, precipitated with ammonia, dissolved in hydrochloric acid and tested with tumeric paper after evaporation almost to dryness.

13. The rare-earth metals were sought in the filtrate from the zirconium phosphate by precipitating with KOH and treating the precipitate with HF, converting into sulfates, precipitating with ammonia, and finally converting into the oxalates. The amounts were extremely small and no concordant results could be obtained.

14. Vanadium, chromium and molybdenum were determined by the Hillebrand (42, p. 185) method on a 5-gm. charge of the soil powder.

Confirmatory qualitative tests for the presence of vanadium and molybdenum were applied as follows: The solution was evaporated to dryness and heated to expel excess of sulfuric acid. The residue was taken up with 2-3 cc. of water containing a few drops of dilute nitric acid. On addition of a few drops of hydrogen peroxide a characteristic brownish tint developed. Certainty as to the identity of molybdenum was established by igniting the sulfide in porcelain and adding a single drop of concentrated sulfuric acid. On heating, a beautiful blue color indicated the presence of molybdenum.

15. The literature on the determination of sulfur as barium sulfate is voluminous. The total sulfur was determined by fusion with sulfur-free sodium carbonate and about 0.5 gm. potassium nitrate, solution of the melt in water, reduction of the manganese with ethyl alcohol and precipitation as barium sulfate. In connection with this determination it is interesting to note that Hillerbrand has shown, contrary to the experience of Stoddart (67), that it is unnecessary to remove the silica. For, if the filtrate measures about 100-200 cc. and is acidified only slightly with hydrochloric acid in the cold, no separation of the silica occurs. This is indeed fortunate for in the case of soils the amount of silica is so high that filtration from the silica would be an almost impossible task. The writer found absolutely no separation of silica by this method. The sulfur given in the tabulated statement was obtained by subtracting from the total sulfur that present as  $SO_3$ , obtained by treating with 100 cc. of 16½ per cent HCl.

16. The loss on ignition has no particular significance. It represents the algebraic sum of a number of changes depending on the temperature employed. With increasing temperature the sulfates are decomposed, all the sulfur being driven off, at which point the alkalis begin to escape. The figures, which are the average of a large number of determinations, are to be regarded as approximations only, for it is difficult to prevent reduction by the organic

matter, even if a low temperature with proper precautions is employed. This fact together with the difficulty of dehydrating the hydrated minerals like mica, limonite and chlorite accounts for the summation being over 100.

17. Boron and fluorine exist in tourmaline and mica respectively, both of which have been found in this soil.

The author's attempts at a quantitative estimation of these elements in this soil have not met with much success. Cook (12) and Cook and Wilson (13, 14), have described without giving details the methods used in estimating boron in soils. The author, however, could not obtain consistent results with the colorimetric method used by these authors or by the method outlined by Hillebrand (42, p. 234-238). The objection found to the colorimetric method was the inability to secure reagents used in the test that gave no color with filter paper dipped in a 0.2-per cent solution of curcumin. The amount present was too small to obtain any success with Hillebrand's method.

18. Two, and sometimes three precipitations were carried out throughout this work. Wherever practicable evaporations and precipitations were made at the boiling point in platinum dishes. The filtrations were made into pyrex glass beakers. Blank determinations were made on all reagents.

#### DISCUSSION OF THE ANALYSIS

Examination of the results indicates that the surface and subsurface soil contain more phosphoric acid than the subsoil. Sodium is much higher in the surface soil than in the other two layers, whereas the subsoil is richest in calcium, magnesium and potassium.

The tendency of iron to work its way down is manifest, the subsoil containing almost twice as much  $\text{Fe}_2\text{O}_3$  as the surface soil. Inasmuch as the  $\text{FeO}$  in the surface soil is more than double the amount in the subsoil, it would appear that the reduction effect due to the larger amount of organic matter in the surface soil offsets the oxidizing effect due to aeration.

The presence of aluminum and iron in large amounts favors the formation of insoluble phosphates of these elements. This may explain why phosphorus is the limiting factor in these soils.

Manganese remains about constant in the surface, subsurface and subsoil.

Titanium oxide ranges from 0.4 per cent to 1.5 per cent in the chief soil types in this country; it is, therefore, relatively low in this soil.

It would appear that the sulfur present is sufficient for present needs; but much of it is combined in such a state, probably in organic combinations, that is not readily soluble in acid.

There is no evidence of the presence of sulfides such as pyrite or marcasite.

It is remarkable that lead and zinc, found in a number of localities in both Siluro-Cambrian and later limestone formations, were not present even in traces in this soil.

Analysis of more or less disintegrated dolomite by Knight (46) showed that 10 per cent of the  $\text{CaCO}_3$  had been removed, and that the  $\text{MgCO}_3$  had remained relatively stable. If the Ca and Mg in this soil had been derived entirely from dolomite we would have a ratio of  $\text{CaO/MgO} = 30.4/21.7$ . It is obvious from the results of the chemical analysis that a large portion of the calcium

must be derived from amphibole, and that a considerable portion of the magnesium is derived from chlorite.

The amounts of chlorine and sulfuric acid are low. If, as experimental evidence seems to indicate, these radicals are necessary for the synthetic processes in plants or as carriers of the alkalies from the soil to the plant, fertilization with chlorides and sulfates may have considerable effect in this experiment.

This soil is naturally rich in potash, and inasmuch as the orthoclase fragments were found by the micro-petrographical determination to be highly kaolinized and broken up, this may explain the reason why potash dressings alone cause little or no crop increase on this soil type.

#### THE MINERALS OF THIS SOIL

Chamberlain and Salisbury (9) and Delage and Lagatu (22, 23) were the first to show that nearly all the common rock-forming minerals are to be found in any ordinary soil, formed in the process of weathering and presenting clear unaltered faces. They recognized, also, the result of metamorphic changes, especially in the feldspars, where alteration products could be recognized in the mineral fragments.

The chemical analysis indicates the total amount of elements present in a soil; the mineralogical analysis, however, helps to show in what way these elements are combined.

Considerable work has been done on the solubility of minerals such as the feldspars, micas, tourmaline, chlorite, orthoclase, muscovite, dolomite, limestone, rutile, quartzite, etc., all of which are measurably soluble in water. For details the reader is referred to the work of W. B. and R. E. Rogers (65), Skey (66), Beyer (4), Daubree (21), Clarke (10, 11, p. 532), Cushman (16, p. 6-7) and Funk (33). Cushman's work is of interest as showing that fresh particles of siliceous rock form, upon contact with and decomposition by water, a colloid or gelatinous substance that forms a film on contact with their surfaces which possesses great power of adsorption.

#### THE AVAILABILITY OF SOME OF THE MINERALS PRESENT IN THIS SOIL

The experimental results on the availability of the various minerals in this soil are not clearly defined.

Cushman (17) called attention to a series of experiments with tobacco seedlings which showed that finely ground orthoclase was nearly as efficient a source of potash plant food as the more soluble potash salts. He emphasizes the necessity for improvements in methods and machines for grinding rock. Priánishnikov (59), Fraps (29, p. 5-16) and Bieler-Chatelan (5) conclude from their experiments that plants assimilate very little potash from orthoclase and microcline even when finely powdered. Fraps showed, however, that they removed from 7 to 13 per cent of the potash from the finely ground micas, biotite and muscovite and Priánishnikov's experiments indicated an assimilation by plants of about 17 per cent of the potash from this mineral. Breazale and Briggs (7) showed that the availability to plants of potash in soils derived from orthoclase-bearing rocks is not increased by addition of lime or gypsum.



The author has been unable to find any experimental work on the availability of the other minerals present in this soil.

#### THE MINERALOGICAL EXAMINATION

The micro-petrographical examinations were made by Professor Arthur P. Honess of the Department of Geology of the School of Mines of the Pennsylvania State College. Examinations were made on separates made by mechanical analysis by the writer. Professor Honess states that owing to the lack of necessary equipment the examination has not been as complete as could be wished.

#### *Surface Soil*

In the surface soil the sand fractions were sieved and examined separately.

*Very fine sand* (0.1 mm.-0.05 mm.). Mixed feldspars about 50 per cent. Quartz about 35 per cent. The microcline occurs in blue-gray cleavage fragments usually fresh and unaltered, although fragments appear at times slightly kaolinized. The remaining 15 per cent represents iron oxides (some specimens of which are magnetic), rutile, chlorite, dolomite and tourmaline, with limonite predominant.

*Fine sand* (0.25 mm.-0.1 mm.). Quartz is present in larger amounts than feldspar. Many fragments of quartz from this sample are highly colored by iron oxide and frequently enclose particles which are brown to black in color and sometimes strongly magnetic. This is probably magnetite or ilmenite. The accessory species are the same as in the very fine sand.

*Medium sand* (0.5 mm.-0.25 mm.). This does not materially differ from the fine sand, except that quartz and dolomite are somewhat more abundant. There is not the variety of accessory species noted in the finer grades.

*Coarse sand* (1 mm.-0.5 mm.). This fraction is composed of about 85 per cent quartz. It occurs as rounded or subangular grains; but are occasionally almost spherical. Iron oxide is present either as independent grains or as patches adhering to quartz. A search was made for accessories by crushing part of the sample and mounting in canada balsam; no new species, however, were observed. The total absence of feldspars is noteworthy.

#### *Subsoil*

*Sand.* The sand fractions of the subsoil were not sieved into the various grades as in the case of the surface soil, but were examined as a whole. About 40 per cent consisted of quartz and 45 per cent of feldspar. Occasionally fragments of dolomite were found; limonite grains are common and also iron stained quartz. Some of the microcline fragments are well preserved, others are slightly colored by iron and show some slight evidence of alteration. As accessory components: Tourmaline, mica and rutile were observed.

#### *Surface, subsurface and subsoil*

*Silts.* The silts were difficult to determine due to their finely divided state. Some attempt has been made to determine the minerals present; complete analysis has not been possible because of the lack of necessary apparatus. A preponderance of feldspar (microcline) was noted with a little quartz and a variety of accessories among which tourmaline, chlorite, amphibole, and sericite were unmistakably present.

The silts from the surface, subsurface and subsoil do not materially differ from one another, all being rich in feldspar and showing many accessory

species. It is of interest to note that the feldspar is essentially microcline. The silts are thus characterized by the absence of altered minerals.

*Remarks on the mineralogical examination*

From the examination of the surface sand fractions, it is seen that the finer the fractions the richer it becomes in feldspar and in the variety of accessory species, which gradually decrease in amounts as the fractions become coarser, because owing to perfect cleavage they are more easily broken into fragments and are consequently less resistant to mechanical forces than quartz. The major portion of the soil is composed of these two minerals.

No apatite was found in the fraction examined. Obviously, therefore, the phosphoric acid in this soil must occur accidentally in other minerals, or has escaped detection as apatite in the clay fractions.

Although zirconium was found by chemical analysis, zircon was not found in the mineralogical examination. However, Professor Honess believes that zircon exists in this soil, but that its identity is masked through admixture with other species. Moreover, it may occur in the amphiboles and pyroxenes which are the same chemically, but differ in their optical properties. They have the general formula  $RSiO_3$  in which R may be calcium, manganese, magnesium, zinc, sodium, lithium, aluminum, zirconium or titanium.

Rutile occurred usually in small needles or twinned forms and in some cases enclosed in quartz.

Tourmaline was found as an accessory in the silts as well as the sands, but in very small quantities. Chemical examination, however, shows that a trace of boron is present.

There was considerable limonite in the surface fractions. Professor Honess estimates that about two-thirds of the accessory species in the very fine sand fraction of the surface soil is limonite, which according to Clarke (11, p. 532) may contain all sorts of admixtures of other substances such as calcium, manganese, potassium, aluminum, sodium, phosphorus.

The mica is present as sericite which is a secondary muscovite.

It is interesting to note that orthoclase, if present as such, must be in very small amounts and highly kaolinized. No fresh unaltered samples were to be seen in any of the fractions. The potash feldspars yield usually potassium carbonate on decomposition. On this matter, Professor Honess has written that, "It has been suggested by some that orthoclase and microcline, being identical in chemical composition and so closely related in physical and optical character, are one and the same species, the microcline character of orthoclase being the result of sub-microscopic twinning. This, according to Iddings, produces a mono-symmetric arrangement of the molecules and orthoclase is then characterized by polysymmetry, and consequently not different in crystallization from microcline. But an investigation of these species as found in soils more certainly points to a different conclusion. They are both abundant in the soils of this region, or, more correctly perhaps,

originated here, and, although the microcline has survived erosion and decomposition through the ages, it is practically left untouched as an erosion fragment, although occasionally fragments appear slightly kaolinized. The orthoclase fragments have long since been destroyed, and its presence now is only indicated by occasional kaolinized skeletal cleavage fragments or its alternative equivalent."

The feldspars, according to Professor Honess, are mixed feldspars of acid character, or albite variety, and may contain sodium, calcium or potash.

Rutile, zircon, tourmaline apparently have little or no plant food value.

#### OCCURRENCE OF TITANIUM, MANGANESE, AND THE RARER ELEMENTS IN PLANTS

The rarer elements are more widely distributed in plants than is commonly supposed which shows that soil conditions are favorable for their accumulation. Inasmuch, moreover, as these elements are present as relatively insoluble compounds, the question arises as to why and how such relatively large amounts become absorbed and assimilated by plants and as to how these elements affect plants.

*Titanium.* According to Czapek (18) Aderholdt found titanium in plants as early as 1852, Wait (74) found it in oak, apple and pear woods, apples, cow peas and cotton seed meal; and Geilman (35) in a number of plants including apples in amount equal to 0.0004 per cent of the air-dried material. Geilman found that it accumulated by preference in the assimilating organs, which appears to confirm Mosea's work (56) that titanic acid is of great physiological importance to plants because it acts as an oxidizing catalyst.

*Barium.* According to Watts (76, v. 1, p. 500), Scheele was the first to mention the occurrence of barium in 1788 in beech trees. It was found by Hornberger (43) also in beech trees. In 1875 Dworzak (26) found 0.089 per cent and 0.26 per cent of barium oxide in the stalk and ear of wheat respectively, that had grown in the Nile Valley. Marsh, Alsberg and Black (51) give the results of a number of determinations of barium in alfalfa, barley, millet and beets collected from various parts of Colorado, Wyoming and Arizona. McHargue (52) discussing the occurrence of barium in the tobacco plant and the sycamore tree, concludes that it is present in combination with organic acids and that since it is contained in the live cells of the higher plants it may function in metabolism. Artls and Maxwell (1) report amounts of barium in the leaves of tobacco, dogwood, cottonwood, cherry leaf, black locust, mulberry, elm, maple, plum, walnut, pear, linden, and box-elder varying from 0.005 per cent BaO in the cottonwood to 1.07 per cent in the mulberry. Headden (39, p. 10-17) found it in alfalfa, corn leaves, pea vines and tobacco grown in Colorado in amounts varying from 0.02 to 0.2 per cent BaO. The writer has found 0.22 per cent BaO in alfalfa grown on the Pennsylvania State College Farm.

*Caesium.* Von Lippman (73) found caesium in the ash of sugar beets.

*Rubidium.* Pfeiffer (57) reported rubidium in sugar beets. According to Watts (76, v. 5, p. 127), it has also been found in coffee, tea and cocoa.

*Vanadium, chromium and molybdenum.* Demarcay (24) identified these elements by the spectroscope in the ash of grapevines, fir and poplar. According to Robinson (62, p. 4), von Lippman found vanadium in the ash of grapevines, sugar beets and various trees.

*Lithium.* Gaunersdorfer (34) and later Tschermak (68) found lithium in a number of plants and Headden (39, p. 10-17) found it in loco weed, alfalfa and tobacco. The writer has found it in alfalfa grown on the Pennsylvania State College Farm.

*Strontium.* Headden (39, p. 10-17) found notable amounts in loco weeds, corn leaves and alfalfa. The writer has found 0.10 per cent SrO in alfalfa grown on the Pennsylvania State College Farm.

*Boron.* Jay (45) found small amounts of boric acid in onions.

*Zirconium.* The author has not been able to find any mention in the literature of the occurrence of zirconium in plants.

Special mention must be made of the work of Robinson (63, p. 9-17), who has carried out extensive investigations, by methods of unquestionable validity, on the relation of some of the rarer elements in soils and plants. Plants grown on soils in which the rarer elements had been found were examined with the following results:

In apples he found barium, strontium, lithium, rubidium and a trace of titanium; in beets, chromium, vanadium, barium, strontium, lithium, rubidium and caesium; in alfalfa, barium, strontium, lithium and rubidium; in beans, lithium and rubidium; in cabbage and bluegrass, a trace of chromium; in corn, timothy and wheat, titanium, rubidium, strontium, lithium and barium; and in the case of timothy, chromium and caesium.

He concludes that lithium is present in traces in all plants examined and that rubidium is present in the majority of cases in amounts ranging from a trace to 0.005 per cent of the dried material, which is larger than the amounts present of the other rare alkalies. Caesium was found in three plants. Chromium was found in amounts ranging from a trace to 0.004 per cent of the material. Vanadium was found in traces in six plants. Barium was found in all cases and strontium in all samples except bean seed. Molybdenum was not found in any samples.

It is of interest to note in connection with Robinson's work that where the plant contains a comparatively large amount of the rare alkalies there is also an abnormal amount of manganese present.

#### THE EFFECT OF THE RARER ELEMENTS ON PLANTS

Very little experimental work has been carried out on the physiological action of the rarer elements on plant tissues.

That lithium has a toxic action is shown by the work of Gaunersdorfer (34), Voelcker (71, 72), Frerking (31) and Ravenna and Zamorani (60). Voelcker found that the chloride and iodide applied at the rate of 2 cwt. per acre tends to check root growth in the case of barley, wheat and corn; in some cases actually destroying a proportion of the plants grown; but they found that the application of the oxide at the same rate had a beneficial effect on wheat and barley. Frerking found the poisonous influence of lithium more pronounced than of magnesium in organisms requiring calcium, but not to calcium-free algae and fungi. Very little work has been done on the effect of caesium; but the work of Lucanus (50) and Voelcker showed that its salts are less harmful than the salts of lithium.

McHargue (53) states that, although it has been known for more than a century that plants are able to extract appreciable amounts of relatively insoluble compounds of barium contained in soils, little has been done to determine whether or not the compounds of this element have any specific function in vegetable economy. He showed that barium compounds in the absence of calcium carbonate are poisonous to plants; but barium carbonate in the presence of calcium carbonate apparently exerts a stimulating influence.

Haselhoff (38) found that strontium had no injurious action, is absorbed by plants, and the results indicate that it replaces calcium when there is an insufficient supply of that ele-

ment. McHargue's (53) results, however, show that  $\text{SrCO}_3$  cannot be substituted for  $\text{CaCO}_3$ . In Loew's (49) experiments on algae, strontium salts injuriously affected the chlorophyll bodies and caused loss of starch-making power and finally death. The physiological effect of rubidium chloride has also been investigated by Loew (48), who in pot culture experiments with Chinese barley, cabbage and spinach found that there was a stimulating action in amounts not exceeding 10 mgm. per 50 kgm. of soil; but when applied at the rate of 50 mgm. per 50 kgm. of soil the stimulating effect was not so great except in the case of cabbage as when small quantities were used.

Hebert (40) tested the effect of the sulfates of chromium, aluminum, magnesium, zirconium, thorium, cerium, lanthanum, etc., on germinated seeds of peas, wheat, rape and the yeasts. The toxicity of the different compounds varied slightly on the different groups, but in general occurred in the following diminishing order: Zirconium, thorium, aluminum, chromium, cerium and magnesium. With regard to vanadium, Ramirez (cited by Robinson, 63, p. 9-17) found that it is absorbed and stored by some plants and causes anomalies in their growth. A review of the literature of the presence and effect of boron in plants is given by Cook (12) and Cook and Wilson (13, 14). It would appear that numerous factors influence the absorption, distribution and action of boron on plants. There is evidence to show that below a certain dilution boron tends to produce stronger roots and shoots. This stimulating action appears to be due to the increase of nitrates. Large amounts are known to be toxic to all plants with the exception of certain fungi.

A fairly complete review of the effect of manganese is given by Dietrick (25).

Summarily it would appear that each ash constituent must be considered as exerting some slight influence on the plant. Though plants may develop normally in a nutrient solution without a given element, it does not necessarily follow that this element, if present, might not exert some influence, for the absence or presence of any particular element might be the deciding factor in determining whether or not the plant reaches full development.

Elements like titanium, manganese, barium, boron, strontium, rubidium, etc. may act as catalyzers.

#### SUMMARY

This introductory paper gives an account of the preliminary work carried out on the soil used in an intensive apple fertilizer experiment conducted in steel rims as an Adams Fund Project. At the present time it is desired to draw attention to the following main facts.

1. The rarer elements vanadium, chromium, molybdenum, zirconium, rubidium, caesium and lithium have been isolated and identified by qualitative and quantitative methods of indisputable validity from a residual soil remote from metamorphic rocks.

2. Positive proof is presented of the presence of barium and strontium, which is the first announcement made of the occurrence of these elements in this phase of the Hagerstown series of soils. The results for  $\text{BaO}$  are believed to be accurate. However, as will be seen, the results for  $\text{SrO}$  are approximate. For complete separation from calcium was not obtained.

3. The calcium and magnesium are derived not only from dolomite, but a considerable portion of the quantity of these elements present are contributed by the silicates amphibole and chlorite respectively. Though this

soil is formed from limestone rocks it contains no  $\text{CaCO}_3$ . Moreover, magnesium is present in larger amounts than calcium.

4. The chief source of potassium in this soil are microcline and the mixed feldspars of acid variety, on the availability of which contradictory evidence has been obtained by different experimenters. Mica is present only as an accessory. The orthoclase has practically all been kaolinized.

5. The existence of iron in the ferrous condition, notwithstanding difficulties in determining the absolute amounts, has been definitely established.

6. The greater portion of the sulfur exists either in the free state or, as seems most probable, in organic combination.

7. All three soil layers contain only a trace of chlorine (chlorides) which is most certainly remarkable for it is almost invariably present in soils to the extent of about .01 per cent.

8. Accurate methods for the determination of boron and fluorine in soils need to be developed.

9. A review of the literature of the occurrence of the rarer elements in plants and of the experimental work on the effect of some of them is given. The question is raised—how do such relatively large amounts of the rarer elements become absorbed and assimilated by plants, and what function, if any, do they possess?

As this experiment develops it is planned to investigate the absorption not only of the main plant food elements, but also of some of these rarer elements and the author hopes that the presentation of the facts in this paper will stimulate work along similar lines by others. It is fully realized that a new set of variables is presented in our already complex soil problems; but it is only by a correlation of all variables that an advance will be made in our knowledge of these formidable biological problems.

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## EFFECT OF CHEMICAL AGENTS ON OXIDATION IN SOIL-FORMING ROCKS AND MINERALS

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### INTRODUCTION

In an investigation on the rate and extent of solubility of soil-forming rocks and minerals it was observed that certain rocks and minerals when treated with certain chemical agents and allowed to stand, developed different colors in these various chemical agents. This color development appeared to be due mainly to oxidation or lack of oxidation of the iron present, and is greatly accelerated by some chemical agents and entirely prevented by others. The results appear to throw much light on oxidation and color in soils and in the earth's crust.

### METHOD AND PROCEDURE

The material studied was banded granite, biotite granite, ferruginous sandstone, hornblende, mica-schist, pegmatite, quartzite, scotch granite, shale, siliceous sandstone, syenite, apatite, amphibole, apophyllite, crysolite, epidote, kaolinite, limonite, oligoclase, orthoclase, prochlorite, pyroxene, quartz, serpentine, siderite, and stilbite. The chemical agents used were  $\text{Ca}(\text{NO}_3)_2$ ,  $\text{KNO}_3$ ,  $\text{KCl}$ ,  $\text{NaCl}$ ,  $(\text{NH}_4)_2\text{SO}_4$ ,  $\text{NaC}_2\text{H}_3\text{O}_2$ ,  $\text{Ca}(\text{OH})_2$ ,  $\text{KOH}$ ,  $\text{NaOH}$ ,  $\text{HCl}$ ,  $\text{HNO}_3$ ,  $\text{H}_2\text{SO}_4$ , citric acid and oxalic acid. Each rock or mineral was treated by all of these chemical agents. With the exception of  $\text{Ca}(\text{OH})_2$  the strength of all these solutions was one-tenth normal.

The rocks and minerals were ground very fine either in an iron mortar or in a grinding machine run by power. By grinding these materials in iron containers they were, of course, contaminated with iron, and it was undoubtedly this free iron which was oxidized or not oxidized under the influence of the different chemical agents, that gave rise to the different colors.

Twenty grams of powdered material and 25 cc. of solution were mixed in a glass tube stoppered with a rubber stopper and allowed to stand for a certain length of time before its solubility was determined. It will be noted that the solid material was submerged in a column of liquid, and that air was excluded from coming in contact with the mixture. It was under these conditions that the color development was first observed. In studying color development more directly and in detail the same general method and procedure were followed,

with few modifications. One of these modifications consisted of adding about 40 cc. of liquid instead of 25 cc. to 20 gms. of solid material and paraffining the mouth of the tube to make absolutely sure that no air entered the tube.

#### EXPERIMENTAL RESULTS

A new or different color developed in most of the rocks and minerals mentioned above. But the thing that it is most desired to report is the fact that the various chemical agents had distinctly different effects upon this color development within any one rock or mineral. Figure 1 indicates the different colors

Bluish grey	Water	Lead grey	Tan	NaCl
White	$\text{CaH}_4(\text{PO}_3)_2$	Grey	Light Tan	$\text{Na}_2\text{C}_2\text{H}_3\text{O}_2$
Brownish yellow	$(\text{NH}_4)_2\text{SO}_4$	Light tan		$\text{HNO}_3$
Greenish grey	$\text{KH}_2\text{PO}_4$	Light grey		$\text{H}_2\text{SO}_4$
Tan	$\text{Ca}(\text{NO}_3)_2$	Greenish grey		HCl
Greenish Grey	Light Tan	Whitish grey		Oxalic acid
Lead grey	Tan	Light grey		$\text{Ca}(\text{OH})_2$
	KCl			

FIG. 1. DEVELOPMENT OF DIFFERENT COLORS IN ORTHOCLASE POWDER TREATED WITH VARIOUS CHEMICAL AGENTS

that developed and the manner of their development, in the case of orthoclase treated with the various chemical agents. This is a typical case of a large number of rocks and minerals tested, except that the intensity or shade of the new color varied according to the native or original color. The orthoclase stood in contact with the respective liquids for over a year.

The color developed in the orthoclase varied considerably with the different chemical agents as well as the rate at which this color developed. Beginning with water, the color was bluish-grey throughout the column; with  $\text{CaH}_4(\text{PO}_3)_2$ , white throughout;  $(\text{NH}_4)_2\text{SO}_4$ , brownish yellow throughout;  $\text{KH}_2\text{PO}_4$ , greenish grey throughout;  $\text{Ca}(\text{NO}_3)_2$ , tan throughout;  $\text{KNO}_3$ , light tan, upper three-

fourths of the column, and greenish grey the lower fourth of the column; KCl, tan upper one-third, and lead grey lower two-thirds; NaCl, tan upper one-third and lead grey lower two-thirds;  $\text{NaC}_2\text{H}_3\text{O}_2$ , light tan upper one-third and grey lower two-thirds;  $\text{HNO}_3$ , light tan throughout;  $\text{H}_2\text{SO}_4$ , light grey throughout; HCl, greenish grey throughout; oxalic acid, whitish grey throughout;  $\text{Ca}(\text{OH})_2$  light grey throughout.

Although the above tendency of color development is typical of a large number of rocks and minerals used, yet in some of the rocks and minerals the shade of the colors varied considerably. This would be naturally expected on account of the difference in the native or original color. The most important thing to bear in mind, however, is that the chemical agents influence this color development and that each chemical agent is usually regular and consistent in producing the same kind of result in all of the rocks and minerals.

Some of these reagents not only help the oxidation to start but even accelerate its rate greatly, while others prevent its inception entirely.  $\text{Ca}(\text{NO}_3)_2$  is the best example for the first group and  $\text{CaH}_4(\text{PO}_4)_2$  for the second. The  $\text{Ca}(\text{NO}_3)_2$  caused a pronounced oxidation in nearly every rock and mineral to which it was added, and at a comparatively rapid rate. In the case of the orthoclase, for instance, the whole column of the material shown in the preceding diagram, was oxidized in about three months, while in such salts as KCl and NaCl it took over fifteen months for only one-third of the column to be oxidized. The  $\text{CaH}_4(\text{PO}_4)_2$  prevented oxidation in almost every rock and mineral to which it was added.

The  $\text{CaH}_4(\text{PO}_4)_2$  not only prevented oxidation in the various rocks and minerals but it even destroyed the oxidizing influence of the various chemical agents, when it was mixed with them. When the rocks and minerals were treated with any chemical agent containing  $\text{CaH}_4(\text{PO}_4)_2$  usually no oxidation appeared. Apparently, the non-oxidizing effect of the  $\text{CaH}_4(\text{PO}_4)_2$  is so dominant that it overcomes and masks the oxidizing effect of the other chemical agents.

Oxidation in water alone was not very consistent. In some of the rocks and minerals it developed, while in others it did not. But wherever it did take place, its rate of development was exceedingly slow.

It will be noted from the preceding description and figure 1 that the oxidation started in every case from the top of the column and worked downward, in a very gradual and regular manner. Evidently the oxidation process was obtaining the oxygen from the liquid column which stood above the solid material. It could not obtain it from the outside atmosphere because the tubes were stoppered air tight.

In some experiments the tubes were left open, but the same general type of results were obtained as when they were closed, except that when they were open the rate of oxidation was slightly greater.

As stated previously the rocks and minerals were ground in an iron mortar and it was the free iron which they acquired during the grinding that was oxi-

dized and gave rise to the tan and yellow colors. While no extensive investigation was undertaken to determine what was exactly the form of this oxidized iron, preliminary examination indicated that it was hydrated ferric oxide. In those chemical agents in which oxidation was prevented, the iron probably remained in the free or ferrous state.

Just how the various chemical agents influence oxidation so differently is not definitely known. The oxidation could not be due to the oxygen in the molecule of the reagents because some of them, such as KCl and NaCl, have no oxygen in their molecule and yet cause oxidation, while others that have oxygen in their molecule such as  $\text{CaH}_4(\text{PO}_4)$ , usually cause no oxidation.

The explanation that seems most plausible and satisfactory is to assume that the oxidation in question is caused mainly by catalytic effects, and that the various chemical agents possess different catalytic powers toward oxidation, and consequently affect this process differently.  $\text{Ca}(\text{NO}_3)_2$  and  $(\text{NH}_4)_2\text{SO}_4$  which induce the most rapid and extensive oxidation may be considered as possessing the most catalytic oxidizing power,  $\text{CaH}_4(\text{PO}_4)_2$ ,  $\text{KH}_2\text{PO}_4$ ,  $\text{CaCO}_3$ ,  $\text{Ca}(\text{OH})_2$  which induce very little or no oxidation may be regarded as possessing very little or no catalytic oxidizing power, while KCl, NaCl,  $\text{NaC}_2\text{H}_3\text{O}_2$  etc. may be classed as intermediate. The manner and character of the oxidation in the various chemical agents seem to strongly support this explanation. On the other hand, it is possible that the effect of the different chemical agents upon the hydrolysis or solubility of the iron may also be responsible for the different manner and rate of oxidation.

#### DISCUSSION

The results reported herein contain at least three important and fundamental facts.

1. The different chemical agents have a very decidedly different effect on the oxidation of iron, both as to rate and extent.
2. The dominant non-oxidizing effect of some of the chemical agents may prevent or mark the oxidizing effect of other chemical agents.
3. The oxidation will take place even when the material is immersed under a deep column of liquid, and even when the freer circulation of atmospheric oxygen is excluded.

These results should tend to throw much light (1) on the oxidation and color of soil at various depths; (2) on the influence of fertilizers on oxidation and color of soils; and (3) on the oxidation and color of the substratum of the earth.

# EFFECT OF REACTION ON GROWTH, NODULE FORMATION, AND CALCIUM CONTENT OF ALFALFA, ALSIKE CLOVER AND RED CLOVER<sup>1</sup>

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In a former investigation (3) it was found that the reaction most favorable for the growth and nodule formation of soybeans and cowpeas is pH 6 to 7, and that corn tolerates a greater acidity than soybeans and cowpeas. In this paper a report is given on the effect of reaction on the growth, nodule formation and calcium content of alfalfa, alsike clover and red clover.

Since a fairly extensive review of the literature is given in a former paper (3) only a few of the more important papers are here reviewed.

The investigations of Salter and McIlvane (17), Hoagland (10), Duggar (6), Joffe (11), and the writer show that many plants require a slightly acid reaction for maximum growth. Under field conditions, however, alfalfa seems to do best on soils which are approximately neutral or which have some calcium in the form of carbonate.

The increase of nitrogen in many legumes from the addition of lime to acid soils has been noted by Lipman and Blair (12, 13), Morse (15) and others. These results were attributed in part to the favorable influence of the lime on the legume bacteria. No doubt this is in part correct, since the investigations of Fred and Davenport (7) and Bewley and Hutchinson (2) show that most species of legume bacteria grow best at a neutral or slightly alkaline reaction.

The influence of reaction on the assimilation of calcium by plants has recently received considerable attention. Morse (15) found that the addition of lime to acid soils increased the percentage of nitrogen in clover, but decreased the percentage of calcium. Shedd (18) noted that the addition of calcium to six different acid soils either as nitrate, oxalate or citrate produced an increase in the calcium content of many plants. He concluded that the soils were not supplying sufficient calcium for normal plant growth. This agrees with the work of Tokenchi (20) who reported that the addition of lime to the soil increased the percentage of calcium in the oat plants grown thereon.

McIntire (14) states that lime-loving plants grow poorly on acid soils, not because of the acid conditions, but because the soil solution is not sufficiently concentrated in calcium carbonate. He concludes that the reaction of the soil is rarely detrimental to higher plants, though it may be to bacteria and fungi. On the contrary, Olsen (16) and Wherry (23)

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<sup>2</sup> The writer wishes to express his appreciation for the helpful suggestions and criticisms tendered by Professors E. Truog and E. B. Fred.

report that there is a correlation between the acidity of the soil and the type of plants grown thereon, which would indicate that the reaction of the soil does have a direct influence on plant growth. The investigations of Salter and McIlvane (17), Duggar (6), Hoagland (10), Conner and Sears (4) and others show that controlled reactions equal to those found in acid soils affect plant growth in the same order as those of the soil.

The investigations of Hartwell and Pember (9), Abbott, Conner and Smalley (1), Conner and Sears (4) and others indicate that some of the unfavorable influences attributed to acid soils are due to soluble aluminum salts. Stoklasa (19), however, noted that small amounts of aluminum salts are beneficial to plants, while large amounts are toxic. He reports that the plant ash always contains aluminium. Denison (5) found only insignificant quantities of soluble aluminum in the water leachings of the acid soils with which he worked. He concluded that any soluble aluminum present in the soil solution is due to the solvent action of the soil acids.

The results of the various investigators indicate that many of the cultivated plants grow best at a slightly acid reaction, while most species of the legume bacteria grow best at a neutral or a slightly alkaline reaction. The reaction of the soil seems to have a considerable influence on the assimilation of calcium by plants and also on the type of plants which grow thereon.

#### EXPERIMENTAL

The plants were grown in quartz cultures in the greenhouse. For containers, 600-cc. percolators were used. The methods and nutrient solution were the same as those described in a previous paper (3). But a somewhat different procedure was followed in adjusting the reactions. This was done by adding a buffer consisting of  $\frac{1}{4}$  gm. of disodium phosphate to a liter of nutrient solution and then adding varying amounts of diluted sulfuric acid or sodium carbonate to portions of the solution to obtain the different reactions of approximately, pH 3, 4, 5, 6, 7, 8, 9 and 10. Sand cultures were used rather than solution cultures, because they furnish a more natural media and are easier to handle, especially for very small seedlings. After the reactions of the sand had become constant by repeated washings with solutions of the desired hydrogen-ion concentration, the seeds were planted and the plants subjected to the different reactions from the time of germination. The sand was inoculated with pure cultures of the proper legume bacteria for two consecutive days as the solutions were renewed. Two weeks after germination the plants were thinned to 6 per culture. When the plants were four months old the sand was washed from the percolators, and notes were made of the growth and the nodule formation.

To determine the amount of calcium in the plant tissue, it was dried, ground to a fine powder, weighed and ignited. The ash was treated with dilute hydrochloric acid. The iron and phosphorus were separated from the calcium by the basic acetate method: a few drops of .05 *N* ferric chloride were added to make sure that all the phosphorus would be precipitated in the form of ferric phosphate. The calcium was precipitated as the oxalate, and determined by titration with standard potassium permanganate.

It was noted that the different reactions pH 3 to pH 10 had no appreciable harmful effects on the germination of any of the seeds, but in all cases the seedlings began to die immediately in the most acid and alkaline cultures. Duplicates agreed throughout. The diurnal changes in reaction of the nutrient solution due to the plants were in proportion to the plant growth. However, at optimum reactions no changes occurred. The behavior of the different plants in changing the reaction of the nutrient solution was practically identical; this change varied from nothing to 0.7 pH.

All the alfalfa seedlings died at pH 3, and only one lived at pH 4, while 5 lived at pH 9, and 4 at pH 5. All the seedlings lived at pH 7 and 8. A very good idea of the growth and size of the plants may be obtained from plate 1. The plants at pH 4, 5 and 6 were somewhat yellow during the entire experiment; those at pH 9 and 10 were green but had few branches. Plants at pH 7 and 8 were decidedly the best. Nodules formed at all reactions at which the plants grew, but they were most profuse at pH 6, 7 and 8. An increase in acidity and alkalinity produced a decrease in plant growth and nodule formation. A decrease in acidity produced an increase in the percentage of calcium in the plant tissue. The comparative growth and the percentage of calcium in the plants at pH 5, 7 and 9 are given in table 1.

At pH 3 and 4 all the alsike clover seedlings died, while only one died at pH 10, and none at any other reaction. The plants which lived grew vigorously at all reactions, except those at pH 9 and pH 10, which were yellow and had slightly brown roots. Nodules formed at all reactions as may be seen from plate 2 which gives the comparative growth of the plants at the different reactions. The best plant growth took place at pH 7 and pH 8; the growth at pH 8 was a trifle better than that at pH 7. The acid reactions, pH 5 and pH 6, showed no injurious effects on the roots of plants though the growth was less at these reactions than at pH 7 and pH 8. The percentage of calcium in these plants increased as the acidity decreased. Table 2 gives the results with the alsike clover grown at the different reactions.

At pH 3 and pH 10 all the red clover seedlings died; an average of three lived at pH 4, five at pH 5 and three at pH 9. Because of an infestation of red spider on the clover, it did not produce so vigorous a growth as did the other two legumes. The comparative growth of this clover at the different reactions is given in plate 3. It will be seen that the roots of these plants are slightly darker than those of the other two series. The dark appearance is due in part to light conditions when the photograph was taken. The roots at pH 4 and pH 10 show a lighter color than those at pH 6 and pH 7. In reality the color was the reverse. The best plant growth was noted at pH 7 and pH 8. Wherever the plants grew, nodules formed. An increase in acidity and alkalinity produced a decrease in plant growth and nodule formation. A decrease in acidity produced an increase in the percentage of calcium in the plant. Table 3 gives the comparative plant growth at the different reactions, and the percentage of calcium in the plants at pH 5, pH 7 and pH 9.



TABLE 1

*The growth, nodule formation, and percentage of calcium of alfalfa at different reactions*

REACTION	NUMBER OF PLANTS		GROWTH	NODULES	DRY WEIGHT, AVERAGE OF 10 PLANTS	CaO
	Initial	Final				
pH					gm.	per cent
3	6	0	Dead	.....	0	....
4	6	1.0	Yellow	Poor	....	....
5	6	3.5	Yellow	Fair	1.92	1.01
6	6	5.5	Fair growth	Good	2.26	....
7	6	6.0	Good	Good	4.10	1.23
8	6	6.0	Good	Good	4.00	....
9	6	4.5	Green but small	Good	2.70	1.36
10	6	1.0	Green but small	Fair	2.20	....

TABLE 2

*The growth, nodule formation and percentage of calcium of alsike clover at different reactions*

REACTION	NUMBER OF PLANTS		GROWTH	NODULES	DRY WEIGHT, AVERAGE OF 10 PLANTS	CaO
	Initial	Final				
pH					gm.	per cent
3	6	0	Dead	.....	....	....
4	6	0	Dead	.....	....	....
5	6	6	Fair growth	Good	1.31	0.97
6	6	6	Good	Good	2.20	....
7	6	6	Good	Good	2.55	1.13
8	6	6	Good	Good	2.90	....
9	6	6	Yellow	Good	1.30	1.38
10	6	6	Yellow	Fair	1.07	....

TABLE 3

*The growth, nodule formation and percentage of calcium of red clover at different reactions*

REACTION	NUMBER OF PLANTS		GROWTH	NODULES	DRY WEIGHT, AVERAGE OF 10 PLANTS	CaO
	Initial	Final				
pH					gm.	per cent
3	6	0	Dead	.....	....	....
4	6	3	Poor	Poor	0.8	....
5	6	5	Fair	Fair	1.64	1.02
6	6	6	Good	Good	2.40	....
7	6	6	Good	Good	2.91	1.07
8	6	6	Good	Good	3.01	....
9	6	3	Fair	Poor	1.20	1.42
10	6	0	Dead	.....	....	....

## DISCUSSION

Strong acidity and alkalinity would not be expected to hinder germination of seeds as much as the growth of the seedlings. The very young seedlings are more sensitive to unfavorable reactions, because of the small quantities of buffer material present. In general, the power of the seedling to withstand unfavorable conditions is in proportion to the size and age of seedling. It is possible for large seeds to supply nourishment to the seedlings several days, thereby keeping them alive; but when the reaction of the medium is very unfavorable, the seedlings soon die after the nourishment in the cotyledons is utilized.

The alfalfa and clover bacteria functioned in nodule formation at a more alkaline reaction than did the soybean and cowpea bacteria, while the limits of nodule formation in the acid range were about the same for all the plants. The results thus far indicate that nodules form wherever root hairs are produced. The formation of only a few nodules on the roots of soybeans and cowpeas outside of the range pH 4 to pH 8 is believed to be due to the absence of root hairs. It is possible for plants to produce a meager growth in solution cultures without the formation of root hairs.

Field observations show that alfalfa responds to lime much more than do soybeans; and the work of Fred and Davenport (7) show that the alfalfa bacteria are less resistant to acidity in pure cultures than the soybean bacteria. But it would be unfair to say that the difference in response of these plants to lime is due more to the influence of the reaction on the respective bacteria than on the plants themselves.

The cause of the difference in effects of the various reactions on the growth of alfalfa and clover is not readily explained because of the number of factors involved. Possibly the most plausible explanation thus far is to be found in the difference in reaction of the cell sap of these plants under normal conditions. The results of Haas (8) and Truog and Meacham (21) show that the reaction of the cell sap of both red and alsike clover is more acid than that of alfalfa. The figures will show clearly that both clovers grew at acid reactions, pH 5 and pH 6, better than did alfalfa, even though one alfalfa seedling survived at pH 4. The results seem to be in keeping with Truog's (22) suggestion concerning the feeding power of plants in an acid medium.

Assuming that most of the calcium in plants acts as a neutralizing and precipitating agent of the by-product in the plant sap from the vital processes, it appears that plants grown at different reactions but with equal amounts of calcium in solution, would have a greater percentage of calcium when grown in the more alkaline range as long as fair plant growth took place, because the acid conditions hinder the precipitation of the calcium salts. Tables 1, 2 and 3 show this to be true. The reaction of the media at which the plants are grown seems to influence the power of the plants to obtain sufficient calcium for normal growth. Since the roots of the plants which sur-

vived the acid reactions, pH 5 and pH 6, showed little or no toxic effects from the acidity, it seems that, due to the acidity, the plants were not getting sufficient nutritives for normal growth. These results show that the reaction of the media in which the plants are grown influences the power of the plant to secure sufficient calcium for normal growth even though the quantities of soluble calcium present are approximately equal. This does not appear to be in accord with McIntire's (14) suggestion that the reaction of the soil is rarely injurious to higher plants.

The influence of reaction may bring about a similar change with other nutritive elements; the effect on nitrogen has already been noted. The results reported with calcium are an average of four determinations.

The very alkaline condition possibly precipitated the iron so completely that the clovers were unable to obtain sufficient amounts of iron for normal growth, and hence, produced a chlorotic effect; this was not apparent, however, with the alfalfa.

The results show distinctly that the reaction of the media in which the plants were grown has a direct influence on the growth and nodule formation of the plants. The reactions which were injurious to growth and nodule formation were within the range of reactions of actual soil solutions and suspensions. Thus the necessity for the proper adjustment of the soil reaction for maximum growth and nodule formation is apparent. Acidity and alkalinity produce toxic effects directly, and also influence the power of the plant to obtain sufficient calcium for normal growth. Further studies on the influence of reaction on plant growth and nodule formation under field conditions are in progress.

#### SUMMARY AND CONCLUSIONS

The effects of different reactions on alfalfa, alsike clover and red clover were studied. These plants were grown in quartz sand cultures using a modified form of Crone's nutrient solution adjusted to the reactions desired. The reactions were kept as constant as possible by the use of two buffers, di-sodium phosphate and sodium carbonate and by renewing the solutions daily. The plants were allowed to grow for four months.

The results may be summarized as follows:

1. Alfalfa and clover seeds will germinate at a reaction which is too acid or too alkaline for the growth of the seedlings.
2. Very young alfalfa and clover seedlings are much more sensitive to acidity and alkalinity than older plants.
3. Red clover seed will germinate and grow to a small extent at pH 4 but has difficulty in becoming established.
4. Alfalfa seedlings will not establish themselves at pH 4, nor will alsike clover; but the alsike clover does better at pH 5 and pH 6 than alfalfa and red clover.

5. Alfalfa and clover produce maximum growth and number of nodules at pH 7 and pH 8, that is, at neutrality or at a slight alkalinity.

6. Alsike and red clover grow better in an acid reaction, pH 5 and pH 6, than alfalfa; alsike clover withstands a very alkaline reaction even better than alfalfa.

7. Nodules form at any reaction at which the plants grow, but the greatest number are formed at or near neutrality.

8. The critical hydrogen-ion concentration differs slightly for the different plants studied; in general it was about pH 4 for all the plants. Red clover has a somewhat higher critical acid concentration than alfalfa and alsike clover.

9. The critical hydroxyl-ion concentration for alfalfa and red clover was about pH 9 to pH 10, while that for alsike clover was somewhat higher.

10. The greater the acidity of the media in which the plants are grown, the less the power of the plants to obtain the basic substance calcium for metabolic processes.

11. The acidity which was found to be injurious to alfalfa, red clover and alsike clover was not any greater and sometimes less than that found in many acid soils.

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#### PLATE 1

ALFALFA GROWN IN SAND CULTURES OF DIFFERENT REACTIONS INDICATED BY NUMBERS  
WHICH GIVE THE APPROXIMATE pH VALUES

The sand was removed and replaced with water in order to show the root growth and nodule formation.

EFFECT OF NEGAL KUGA ON SOME LEGUMES  
 D. C. BRYAN

PLATE I

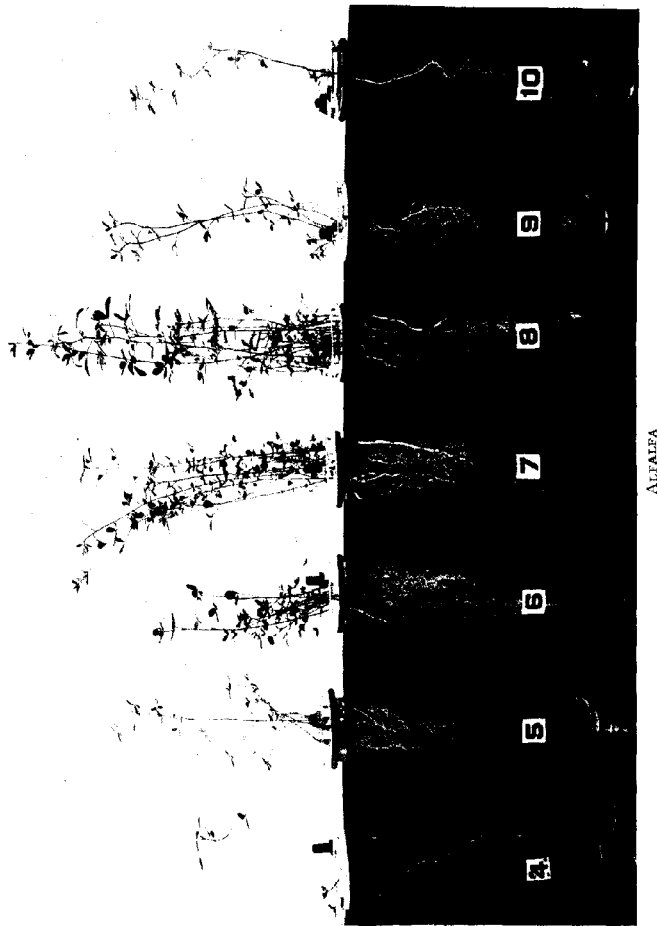


PLATE 2

ALSIKE CLOVER GROWN IN SAND CULTURES OF DIFFERENT REACTIONS INDICATED BY  
NUMBERS WHICH GIVE THE APPROXIMATE pH VALUES

The sand was removed and replaced with water in order to show the root growth and  
nodule formation.

EFFECT OF REACTION ON SOME LEGUMES  
O. G. LUKAN

PLATE 2

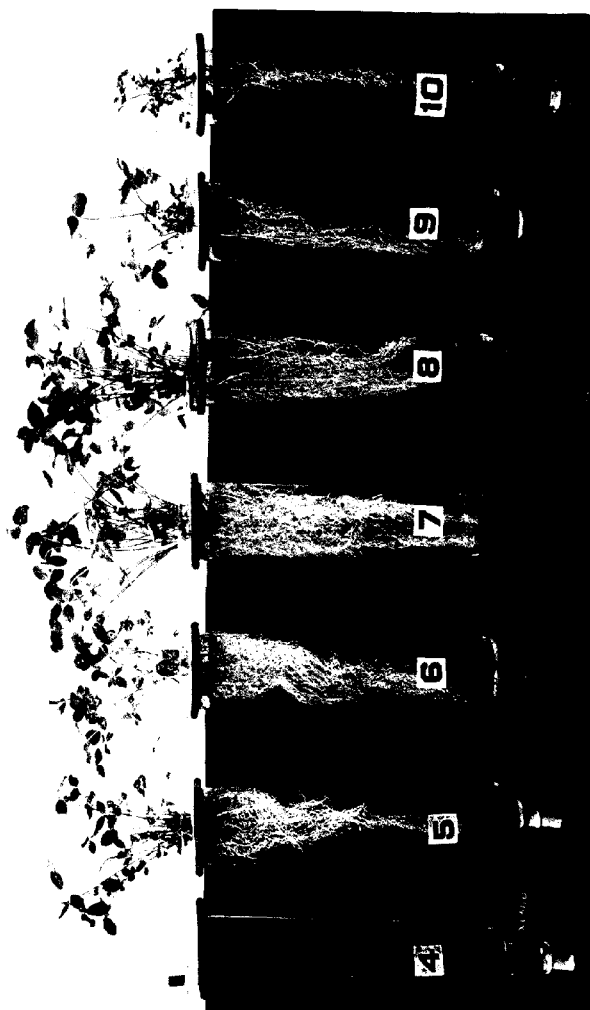




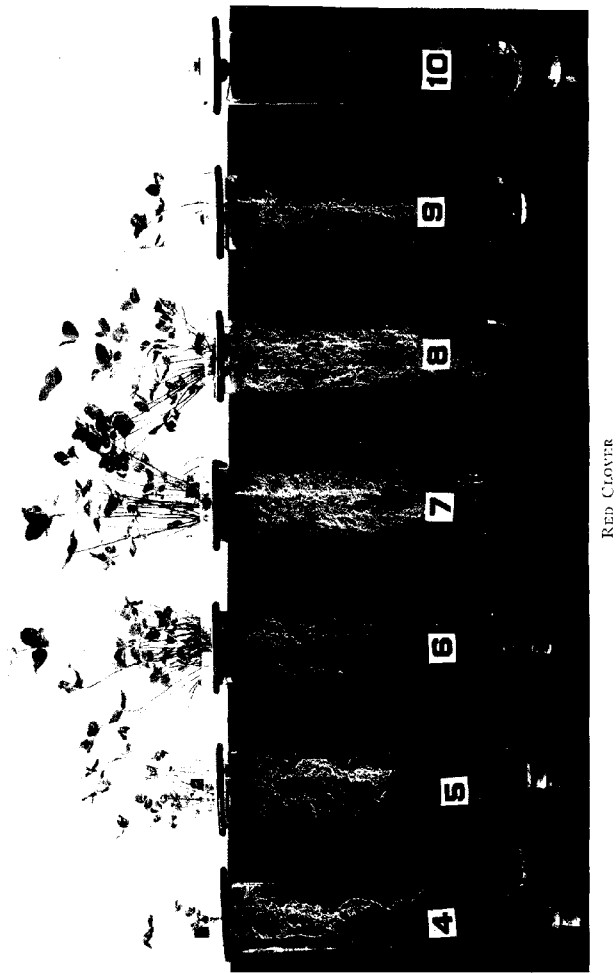
PLATE 3

RED CLOVER GROWN IN SAND CULTURES OF DIFFERENT REACTIONS INDICATED BY NUMBERS  
WHICH GIVE THE APPROXIMATE pH VALUES

The sand was removed and replaced with water in order to show the root growth and  
nodule formation.

EFFECT OF REACTION ON SOME LEGUMES  
O. C. BRYAN

FIGURE 2



RED CLOVER



## EFFECT OF ACID SOILS ON NODULE-FORMING BACTERIA<sup>1</sup>

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It is generally recognized that the nodule bacteria are sensitive in varying degrees to acidity and hence some investigators (4) have suggested classifying the different species of nodule bacteria according to their critical hydrogen-ion concentration. This arrangement is based entirely on laboratory studies and does not prove that the legume bacteria will behave similarly in acid soil. Bewley and Hutchinson (1) reported that a number of species of nodule bacteria were either killed or rendered inactive in definitely acid soils, but the degree of acidity was not given. Whether or not these bacteria are killed in acid soils, and at what reaction they cease to produce nodules on the host plants are questions of considerable importance from both a practical and a scientific standpoint. The present investigation was undertaken to study the effect of acid soils of known reactions on a few of the important nodule-producing organisms.

Two-hundred-gram portions of five different acid soils ranging from sand to fine sandy loam and silt loam were treated with varying amounts of lime to obtain a series of different reactions for each soil. Portions of the soil so treated were placed in large cotton-stoppered Pyrex glass tubes and inoculated separately with suspensions of alfalfa, red clover, and soybean bacteria. The tubes were kept at room temperature and optimum moisture was maintained by frequent additions of sterile water.

At the end of 75 days heavy suspensions of each soil representing the different reactions were made in sterilized water, and the suspension from each was poured upon sand cultures of alfalfa, red clover, and soybean seedlings previously treated to free them from legume bacteria. The seedlings were allowed to grow for 5 to 7 weeks during which time sterilized water was added from time to time to keep the moisture favorable for growth. The results of nodule formation on these plants for each kind of soil and for each reaction are given in table 1.

The results presented in the table agree with field observations; namely, ~~that~~ the nodule bacteria are killed in acid soils of a certain degree of acidity

<sup>1</sup> Approved for publication by the Director of the Wisconsin Agricultural Experiment Station.

<sup>2</sup> The writer wishes to express his appreciation for the helpful suggestions and criticisms tendered by Professors E. B. Fred and E. Truog.

TABLE 1

*The ability of alfalfa, red clover and soybean bacteria to produce nodules after 75 days incubation in acid soils*

TEXTURE	LIME APPLIED PER ACRE	RE- ACTION  <i>pH</i>	NODULE FORMATION FROM INOCULATION WITH THESE SOILS		
			Alfalfa plants	Red clover plants	Soybean plants
Sand .....	0	5.8	*	*	*
	1	6.1	*	*	*
	2	6.5	*	*	*
Fine sandy loam .....	0	4.9	None	*	*
	1	5.3	*	*	*
	2	5.8	*	*	*
Silt loam .....	0	5.4	*	*	*
	1	5.7	*	*	*
	2	6.0	*	*	*
Silt loam .....	0	4.5	None	None	*
	1	5.1	Few nodules	*	*
	2	5.5	*	*	*
Silt loam .....	0	3.4	None	None	None
	1	4.2	None	None	*
	2	5.3	*	*	*

\* Asterisk denotes presence of nodules.

TABLE 2

*The effect of acid soils on the nodule-producing power of alfalfa bacteria*

TEXTURE	TREATMENT	RE- ACTION	NODULES
		<i>pH</i>	
Fine sand .....	Control	4.86	None
	Potassium chloride	4.51	None
Peat .....	Control	4.70	None
	Lime	5.4	Nodules
Silt loam .....	Control	5.36	None
	Sodium phosphate	6.1	Nodules
	Lime	5.96	Few nodules
Sand .....	Control	4.9	None
	Lime	5.3	Nodules

within a few months. The limiting hydrogen-ion concentration for the alfalfa bacteria was at pH 5.0; for the red clover, pH 4.5 to 4.7; for the soybean, pH 3.5 to 3.9 regardless of the type of soil. The critical hydrogen-ion concentration for these bacteria in soils agrees with that for the same nodule bacteria in pure culture as reported by Fred and Davenport (4).

In the present investigation nodules were formed on the soybeans at a greater acidity than they were in solution cultures of soybeans in a previous investigation (2). This difference may be accounted for by the absence of root hairs in the very acid range of the solution cultures. On the other hand, alfalfa and clover failed to form nodules at so high a degree of acidity as was noted in another investigation (3) with quartz cultures of these two plants. Possibly the very large number of bacteria used in inoculating these quartz cultures resulted in some of the bacteria entering the root hairs before they had become injured by the acid. The legume bacteria after they have entered the root hairs of the host plant are either better protected from, or more resistant to the injurious effect of acids in the medium in which the host plant is growing.

Microscopic studies were made of alfalfa, red clover, and soybean bacteria which were grown on agar slants of different hydrogen-ion concentrations. It was found that an acid reaction reduced the normal size and retarded the life cycle of these bacteria.

Since there was a fairly definite limit for nodule formation determined by the reaction of the soil in which the bacteria had been kept, it was thought well to note the effect of several other acid soils on the formation of nodules on alfalfa. Four soils (fine sand, sand, silt loam, and peat) were being tested for fertilizer requirements of alfalfa and had been planted to this crop in 2-gallon jars; suspensions of alfalfa bacteria had been added to each jar. Several of these soils produced only small seedlings, some of which were yellow indicating that the plants had not become inoculated. Examinations were made of these plants for nodules, and the reaction of the respective soils was determined electrometrically. The results are given in table 2. The limiting reaction for nodule formation with all the different soils was about pH 5 for the alfalfa bacteria. There was one soil, silt loam, which failed to produce favorable conditions for nodule formation at pH 5.36. The data presented, however, are not sufficient to attribute the absence of alfalfa nodules at pH 5.36 to soil type.

The preceding experiments bring out the need for liming acid soils and frequent inoculation of them. The detrimental action of soil acids on the legume bacteria no doubt accounts for the failure of some crops to become inoculated although the seeds have been treated with the desired bacteria.

#### SUMMARY

The ability of alfalfa, red clover and soybean bacteria to live and produce nodules, 75 days after being placed in acid soils of different degrees of acidity, was studied with results as follows:

1. The alfalfa bacteria were killed at about pH 5; the red clover bacteria at pH 4.5 to 4.7; and the soybean bacteria at pH 3.5 to 3.9.
2. The critical hydrogen-ion concentration in the soil is approximately the same as in pure cultures.
3. The texture of the soil seems to have no appreciable influence on the critical hydrogen-ion concentration for the bacteria studied.
4. The necessity of frequent inoculation and liming of acid soils is indicated.

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## ACID PHOSPHATE PRODUCTION BY THE LIPMAN PROCESS: II. BUILDING UP SULFUR-FLOATS-SOIL MIXTURES WITH A HIGH CONTENT OF TOTAL AND SOLUBLE PHOSPHATE<sup>1</sup>

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The slowing down of the conversion of insoluble phosphates with increase in bulk of the floats in the sulfur-floats-soil mixtures and the speeding up of conversion with diluting the mixtures, suggested the idea of gradual additions of floats and sulfur in the proper proportions to the dilute mixtures in order to build up the concentrated mixture.

### EXPERIMENT 2: INITIAL STAGE

The following mixture was made up: 75 gm. greenhouse soil, 15 gm. floats and 10 gm. of sulfur. Of this mixture, 800 gm. was placed in each of 2 pots, the layer being 4-5 inch deep. The moisture-content was kept at 50 per cent of the total moisture-holding capacity until the hydrogen-ion concentration went down to pH 2.8. It was then raised to 60 per cent of the total moisture-holding capacity. Experiment 1 described in the first paper of this series suggested such a procedure. The sulfur was increased with a definite idea in mind based on the principles of the mass law. This law tells us, that the velocity of any reaction depends upon the mass of the active ingredients involved and is at any moment proportional to the molecular concentration of the reacting components and a constant which is characteristic of the chemical nature of the reacting substances. The increase of sulfur increased the sphere of activities of the sulfur-oxidizing organisms. The greater mass of sulfuric acid thus formed thus increased the velocity of the reaction involved in the transformation of the insoluble phosphates.

Table 1 gives the course of reaction and transformation of the insoluble phosphates.

The rapid increase of the available phosphates as the hydrogen-ion concentration of the water extract increased, the large amount of sulfur oxidized, and the still greater increase of the hydrogen-ion concentration after all of the insoluble phosphates had been converted into soluble form are significant

<sup>1</sup> Paper No. 104 of the Journal Series, New Jersey Agricultural Experiment Stations, Department of Soil Chemistry and Bacteriology.

This paper will appear in *RUTGERS COLLEGE STUDIES*, vol. 1.



TABLE 1

*Course of reaction and transformation of insoluble phosphates in dilute sulfur-floats-soil mixtures*

AGE OF CULTURE	CULTURE 1		CULTURE 2	
	Reaction	Available phosphorus per 100 gm. of mixture	Reaction	Available phosphorus per 100 gm. of mixture
<i>weeks</i>	<i>pH</i>	<i>mgm.</i>	<i>pH</i>	<i>mgm.</i>
1	5.0	31.6	5.0	32.7
2	3.4	41.8	3.4	43.9
3	3.0	72.7	3.0	73.4
4	2.8	99.4	2.8	101.3
5	2.8	129.7	2.8	128.9
6	2.6	179.9	2.6	183.6
7*	2.2	186.7	2.2	187.4
8	1.6	183.4	1.6	182.7

\* After the seventh week a one per cent HCl extract showed that 6.47 gm. of sulfur had been oxidized.

and instructive phenomena. First, they illustrate the fundamental reactions involved in the formation of soluble phosphates when rock phosphate is treated with sulfuric acid. Second, they hasten the accumulation of phosphoric and sulfuric acids, which is utilized in the later stages of building up the composts. The reactions of transformation of insoluble phosphates to the soluble form belong to the type of reactions of heterogeneous system. In such heterogeneous system the speed of the reaction is a function of a greater number of variables than in the case of a homogeneous system. According to Kazakov (2, 3), there are factors which are common to both systems and they are:

1. Concentration of the reacting mass
2. Temperature of the reacting medium
3. Amount of contact of the reacting substances
4. Speed of diffusion of the reacting substances
5. Catalytic agents

Besides these factors we have others in a heterogeneous system where solid solution phases occur. These are:<sup>2</sup>

6. Size of contact surface<sup>3</sup>
7. Chemical composition of the solid phase
8. Physical properties of the solid phase
9. Influence of formation of a solid phase as a result of the reaction

<sup>2</sup> Some parts of Kazakov's work have been reported already but are treated here more extensively for the sake of completeness.

<sup>3</sup> The size of the particles of the rock in the manufacture of acid phosphate has a tremendous influence. Theoretically, all other conditions being equal, the speed of solution of a solid in a liquid is proportional to the contact surface, and in spherical bodies (as we suppose in fine floats) the surface is proportional to the square of the radius. Particles with a radius of 0.1 mm. will dissolve twenty-five times as fast as particles with a 0.5 mm. radius.

Factors 7 and 8 have a tremendous influence on the speed of the reaction and they are the least known, since the chemical make-up of the rock phosphate is still obscure. Kazakov (2, 3) formulates the reactions involved in the formation of soluble phosphates as follows:

TREATMENT	RESULTS	
	Liquid phases	Solid phases
H <sub>2</sub> SO <sub>4</sub> added in excess . . . .	H <sub>3</sub> PO <sub>4</sub> , H <sub>2</sub> SO <sub>4</sub> , sulfates of Ca, Al, Fe	CaSO <sub>4</sub> ·2H <sub>2</sub> O
Close to optimum . . . . .	H <sub>3</sub> PO <sub>4</sub> , sulfates of Ca, Al, Fe	CaSO <sub>4</sub> ·2H <sub>2</sub> O
Optimum . . . . .	H <sub>3</sub> PO <sub>4</sub> , sulfates of Ca, phosphates of Al and Fe	CaSO <sub>4</sub> ·2H <sub>2</sub> O
Not enough acid . . . . .	H <sub>3</sub> PO <sub>4</sub> , sulfates of Ca, phosphates of Ca, Al and Fe	CaSO <sub>4</sub> ·2H <sub>2</sub> O and part of undissolved phosphate

In the sulfur-floats-soil mixtures the amount of sulfuric acid formed at any moment was small and in the presence of large amounts of tricalcium phosphate there was no accumulation of acid. Besides these purely chemical considerations the biological factor should not be lost sight of. As yet it is not established at what concentration of the soluble substances formed by the sulfur-oxidizing organisms, become toxic and hinder the activities of the organisms. But leaving this unknown factor aside for the present, let us follow up the scheme of reactions a little further. In the system of sulfur-floats-soil mixtures the liquid phases possible are H<sub>3</sub>PO<sub>4</sub>, sulfates and phosphates of calcium. In the solid phase we find gypsum and undissolved phosphates. Undoubtedly the liquid phase of the phosphates is in the form of primary phosphates. These react with the tertiary phosphates and bring them in solution. An accumulation of the soluble phosphates lowers the concentration of the soluble CaSO<sub>4</sub>·2H<sub>2</sub>O, after an initial increase, as shown in figure 1, which is based on Kazakov's data (2, 3). It is very likely that the solubility of the gypsum is the retarding factor in the sulfur-oxidizing activities of the organisms, since high concentrations of phosphates do not seem to effect their activities. This occurs when the mixtures reach a pH value of 2.8-2.6. At this point the transformation of the phosphates gains momentum, phosphoric acid accumulates and a rapid oxidation of sulfur may be noticed. If it were only possible to keep the mixtures under such conditions, adding gradually small amounts of insoluble phosphates the process might be shortened to such a time period that it would be practical on a factory scale. Tables 1 and 2 illustrate the second phenomenon, namely the accumulation of phosphoric and sulfuric acid which is utilized in the building up of concentrated compost. The material from this experiment was used as a base mixture and will be referred to as "stock culture."

## EXPERIMENT 3

From the stock culture of experiment 2 the following mixtures were made up:

CULTURE NUMBER	CULTURE MATERIAL	GREENHOUSE SOIL	FLOATS	SULFUR
	gm.	gm.	gm.	gm.
1	50	25	15	5
2	50	15	25	10
3	50	10	25	15
4	50	0	25	25
5 and 6	150		25	10
7 and 8	150		45	15
9 and 10	150		65.5	22.5
11 and 12	150		25	25

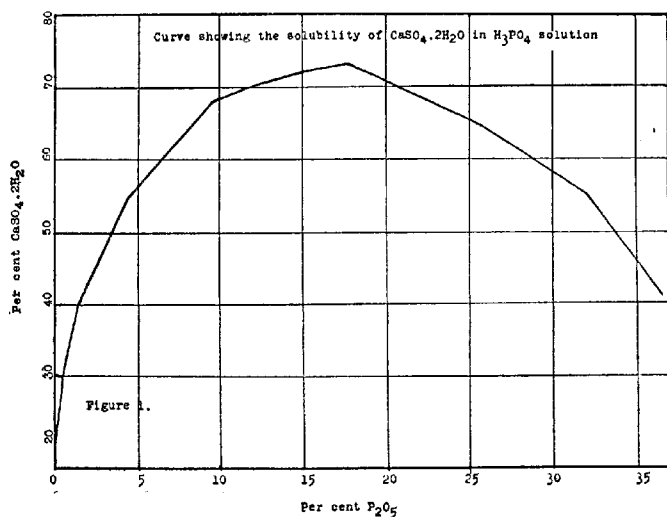


FIG. 1. SOLUBILITY OF  $CaSO_4 \cdot 2H_2O$  IN  $H_3PO_4$  SOLUTION

The cultures were placed in tumblers, except 7, 8, 9 and 10 which were kept in small pots. They were incubated at a temperature of 25 to 28°C. Table 2 gives the results of the experiment.

These data show that the inert material, greenhouse soil, is of importance in speeding up the activities of the sulfur oxidizing organisms. Culture 1 may be taken as an illustration. The beneficial effect of organic material on sulfur oxidation is not due to the importance of the nitrogen in the organic matter in the metabolism of the sulfur oxidizing organisms as Rudolfs (5) attempted to show. The organic matter seems to serve as a temporary storage

place for the acid produced by the sulfur-oxidizing organisms, thus removing the sulfuric acid as fast as it is formed. That organic substances have no direct beneficial effect on the activities of the sulfur-oxidizing organisms is pointed out in discussing the effect of organic substances on *Thiobacillus thiooxidans* (1). The data on cultures 1, 2, 3 and 4 illustrate how the organic matter influences the solubility of the phosphates which serve as a measure of the sulfur oxidized. Cultures 2 and 4 contained the same amounts of rock phosphate, and although culture 4 had more sulfur it was behind in solubilizing capacity. The amount of greenhouse soil added to culture 2 is the factor which made it superior to culture 4. An inspection of the data after 7 weeks of

TABLE 2

CULTURE NUMBER	AFTER 1 WEEK			AFTER 4 WEEKS		AFTER 7 WEEKS			AFTER 14 WEEKS		
	Reaction	P in 1 gm. of mixture	Citrate soluble P in 1 gm. of mixture	Reaction	Citrate soluble P in 1 gm. of mixture	Reaction	P in 1 gm. of mixture	Citrate soluble P in 1 gm. of mixture	Reaction	P in 1 gm. of mixture	Citrate soluble P in 1 gm. of mixture
	pH	mgm.	mgm.	pH	mgm.	pH	mgm.	mgm.	pH	mgm.	mgm.
1	3.8	30.4	12.99	2.8	18.76	2.1	27.8	25.96	1.8	27.6	26.4
2	4.4	45.6	10.36	2.8	20.36	2.6	42.0	25.74	2.4	41.7	30.04
3	4.4	44.8	9.56	2.8	19.52	2.6	43.1	23.3	2.4	42.8	29.56
4	4.2	43.2	9.84	3.0	13.88	2.8	41.6	20.68	2.4	40.0	25.2
5	3.0	41.8	14.4	2.8	22.56	2.5	38.2	25.16	1.8	38.6	26.48
6	3.0	41.8		2.8	23.36	2.5	38.2	25.16	1.8	38.8	27.68
7	3.8	44.3	13.36	3.0	16.88	2.6	39.4	24.0	2.4	37.85	29.76
8	3.8	44.3		2.8	20.8	2.4	39.2	25.2	2.2	37.73	30.07
9	3.6	56.8	13.46	3.0	14.64	2.6	51.8	24.0	2.2	51.5	28.8
10	3.6	56.8		2.8	19.52	2.6	51.7	24.0	2.2	51.5	29.56
11	3.2	40.1	14.4	2.4	21.0	2.2	36.2	24.0	1.8	35.7	27.96
12	3.2	40.1		2.4	21.48	2.2	36.2	24.2	1.9	36.1	25.92

incubation shows that in culture 1 practically the entire amount of insoluble phosphates has been converted into soluble forms. But it is important to note that the total amount of phosphorus was only 2.7 per cent, and the bulkiness of such mixtures, from a commercial standpoint, will bar it from practical application. Cultures 11 and 12, because of the large amount of the original culture material, indicate the influence of inoculation material. On the other hand cultures 9 and 10 show that the process has tremendous possibilities. Close to 50 per cent of the total phosphorus was solubilized, notwithstanding the high concentration of total phosphorus. In terms  $P_2O_5$  the total phosphates amounted to 12 per cent of the mixture. It is worth mentioning that after the next 7 weeks of incubation only 60 per cent of the

total phosphates had become soluble in mixtures 9 and 10. Still more prolonged incubation brought into solution more of the insoluble phosphates. Thus within 24 weeks over 75 per cent of the total phosphorus was made soluble; but most of the cultures were not incubated that long. After the tenth week of incubation, the next step of building up the total percentage of phosphates was begun. This step is discussed under experiment 4.

#### EXPERIMENT 4: SECOND AND THIRD STAGES

Culture 1 received a mixture of 4 parts of rock phosphate to one part of sulfur, whereby the total phosphate content should not have been any greater than 6 per cent. Cultures 2, 3 and 4 were mixed, air dried and after 5.5 weeks a 5 to 1 mixture of rock phosphate and sulfur was added to make the total phosphorus content about 6 per cent; this was called culture A. Cultures 5, 6, 7 and 8 were mixed and a 4 to 1 mixture of rock phosphate and sulfur was added to make a total phosphorous content of not more than 8 per cent; this was called culture B. Cultures 9, 11, and 12 were mixed, air dried, kept for 5 weeks and then rock phosphate was added to this mixture to make a total phosphorous content of not more than 6 per cent; this was called culture C. No sulfur was added because of the excess of sulfur added to these cultures originally.

Table 3 gives the results of this experiment.

An analysis of these results shows outstanding features which should lead to definite conclusions. A few points are, however, interesting. First, the cultures with an original high organic-content showed more efficient sulfur-oxidizing capacity. Culture 1 may again be cited as an illustration. After 3.5 weeks of incubation in the second stage 55 per cent of the phosphates out of a total 5.7 per cent P were made soluble. A new addition of rock and sulfur was made and after another 6 weeks' incubation more than 50 per cent of a total of 6.32 P or 15 per cent  $P_2O_5$ , became soluble. Second, where the concentration of the phosphates becomes higher the second stage is prolonged; culture B is an illustration. The amount of soluble phosphorus approached 56 per cent of a total of 6.98 per cent of phosphorus or 16 per cent  $P_2O_5$ , but the period was prolonged to 20 weeks. Culture C with a slightly lower total of P shows 64 per cent of soluble P in 18 weeks. It is possible that the more rapid activity of this culture was due to the new method of handling the culture. It was air dried to see whether any of the original soluble phosphates would revert upon drying; analyses showed no signs of reversion. The short period of air drying seems to have no effect on the sulfur-oxidizing organisms; if anything it stimulated the activities, a phenomenon well known in soil-microbiology. The failure of culture A to show activity is due to the fact that this culture was subjected to intermittent air dryings and moistening.

The investigations of the sulfur-floats-soil mixture were conducted with the aim to reach a mixture with a high total concentration of phosphorus and as much available phosphorus as possible in the shortest period possible. Thus

far mixture 1 was the best, and the results were accomplished in three stages: the first stage 7 weeks, the second 3.5 weeks, the third 8 weeks; the last stage probably could have been shortened 2 weeks, since the addition of the rock phosphate in the third stage could have been made two weeks earlier. Even then the total period for building up mixture 1 was 18.5 weeks, besides the time necessary for the preparation of the original culture. Combining the latter it took 24 weeks to get a mixture of 15 per cent total  $P_2O_5$  with 50 per cent of it soluble. However, if these results are compared with those of McLean (4), Rudolfs (5) and the earlier work of the author they become significant. McLean (4) stated: "In regard to the question as to whether sufficient  $P_2O_5$

TABLE 3

*Course of conversion of insoluble phosphates into soluble form in the second and third stages of the Lipman process*

INCUBATION PERIOD	CULTURE I			CULTURE A			CULTURE B			CULTURE C		
	Reaction	P in 1 gm. of mixture	Citrate soluble P in 1 gm. of mixture	Reaction	P in 1 gm. of mixture	Citrate soluble P in 1 gm. of mixture	Reaction	P in 1 gm. of mixture	Citrate soluble P in 1 gm. of mixture	Reaction	P in 1 gm. of mixture	Citrate soluble P in 1 gm. of mixture
weeks	pH	mgm.	mgm.	pH	mgm.	mgm.	pH	mgm.	mgm.	pH	mgm.	mgm.
0.0	2.6	58.48	20.11	3.4	60.1*	17.0	3.6	77.6	20.9	4.0	58.6	23.0
3.5	2.8	57.45	31.93	...	...	...	2.8	73.1	24.8	...	...	...
5.5	3.6†	66.35	15.00	2.8	62.6	21.2	...	...	...	...	...	...
9	...	...	...	...	...	...	...	...	...	2.4	57.4	33.9
11.5	2.8	63.25	31.9	...	...	...	2.3	...	32.45	...	...	...
18	...	...	...	...	...	...	...	...	...	2.4	57.2	36.95
20	...	...	...	...	...	...	2.4	69.8	39.19	...	...	...

\* The low figure for the total P is due to some error which was discovered too late to permit making a redetermination.

† Since after 3.5 weeks of incubation more than 50 per cent of the phosphorus was soluble, more rock and sulfur was added to make up a mixture with a total phosphorus content about 7 per cent.

is made available to warrant the employment of concentrated composts, it must be admitted that the data at hand do not justify an affirmative answer. The present data do prove, nevertheless, that there is a possibility that some chemical treatment may be worked out, in which a stimulation of the bacterial activities will be brought about, which will in turn speed up the sulfonation processes.<sup>27</sup> The citation shows the appreciation of the difficulties and a hopeful vision. The achievement of a concentrated compost is an accomplished fact, although the incubation period is still somewhat long. In experiments yet to be reported, another step forward has been made toward the solution of the problem of making acid phosphate by the Lipman process.

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# MICROBIOLOGICAL ANALYSIS OF SOIL AS AN INDEX OF SOIL FERTILITY: IV. AMMONIA ACCUMULATION (AMMONIFICATION)<sup>1</sup>

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When the physiological methods for measuring the activities of soil micro-organisms were first suggested, they were looked upon as giving a biological basis for measuring soil fertility. A great amount of work has been done along this line and we are still uncertain whether these soil biological activities as determined by the present methods actually interpret soil fertility. Of these activities, the so-called ammonification, together with nitrification and nitrogen-fixation occupy the leading place. It is the purpose of this as well as the following papers to compare the various methods in use and to establish whether or not the phenomena in question can actually be used as soil biological functions. No attempt will be made to review the extensive literature, but attention will be called to some of the outstanding investigations having a direct bearing upon the problem under consideration. A detailed study of the literature on ammonification up to 1910, is given by Löhmis (34).

The measurement of ammonia formation or rather ammonia accumulation from a certain organic substance, as a source of nitrogen, usually a complex protein, is used by the soil bacteriologist for the study of four groups of phenomena which may be outlined as follows:

1. AVAILABILITY OF NITROGENOUS FERTILIZERS. Valuable information has been obtained from the study of ammonia accumulation from various organic and inorganic materials, in simple or mixed fertilizers, which can serve as an indication of the speed with which these materials will be broken down in the soil with the liberation of ammonia when used for fertilizing purposes [Lipman and associates (27, 31)]. C. B. Lipman and Burgess (22) found, however, nitrification of organic nitrogenous materials a better index of their availability than ammonia accumulation. Lathrop (20) pointed out that in the decomposition of protein materials, such as dried blood, intermediate compounds are formed which are undoubtedly beneficial to plant growth. He maintained, therefore, that ammonification is not a complete index of the availability of nitrogen in organic fertilizers. This applies, of course, to nitrification as well.

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2. COURSE AND RAPIDITY OF DECOMPOSITION OF NITROGENOUS ORGANIC MATTER. When ammonia is used as an index of the amount of organic matter decomposed, important information can be obtained as to the influence of various environmental conditions, such as moisture, aeration, physical and chemical condition of medium, presence and absence of other nitrogenous and non-nitrogenous organic and inorganic substances, influence of stimulants, etc., upon the decomposition of the organic matter. As an important example, we might mention the decomposition of manure by microorganisms, a review of which is given by Löhnis (34, p. 580). This subject has been recently taken up by Conn and Bright (9). In this case, however, especially if a pure protein is used, a clearer understanding should be had of the mechanism of ammonia formation and of the other processes, leading to the disappearance of ammonia, such as nitrate formation and ammonia assimilation by various soil bacteria, fungi and actinomycetes. For the decomposition of organic residues in the soil, containing only small amounts of nitrogen, the  $\text{CO}_2$  production is a much better index.

3. COMPARISON OF PURE CULTURES OF MICROORGANISMS. Since ammonia can be used as an index of the rapidity of decomposition of nitrogenous organic materials, various microorganisms can thus be compared as to their ability to decompose these organic materials and relative speed of the reaction. This may not be necessarily of direct practical importance, but it is of theoretical importance, since it helps us to interpret the rôle of the various groups of soil microorganisms in the decomposition of organic matter in the soil and allows us a better insight into the activities of these various groups of microorganisms.

4. COMPARISON OF SOILS. Soils, different in nature and changed by a difference in treatment, may be compared as to their fertility, by their efficiency of accumulating ammonia from definite organic materials rich in nitrogen.

It is this last phenomenon that has been made the subject of a large part of the work on ammonification and which concerns us here. The question is whether a difference in ammonia formation from a certain organic material between two soils indicates a difference in fertility or is it merely a result of different chemical, physical, biological and merely chance factors having nothing to do with soil fertility?

The methods used in determining the ammonifying capacity of the soil are divided into two groups: First, solution methods, whereby a definite quantity of soil is introduced into a solution of a definite protein and the ammonia formed determined after a certain period of incubation and, second, soil methods in which the protein is added directly to the soil to be tested. The action of bacteria on proteins results in the formation of albumoses, peptones, amino acids, acid amides and ammonia as the final product of the processes of hydrolysis and deamination. By treating the culture (solution or soil) with  $\text{MgO}$  and boiling, the ammonia and acid-amid nitrogen are driven over as ammonia. By the aeration method only the ammonia nitrogen is determined.

The fact that soil organisms, both bacteria and fungi, are active in the breaking down of various proteins with the formation of ammonia was pointed out in 1893 by Müntz and Coudon, Marshal, and soon afterward by other investigators. Chester (8) determined the ammonifying efficiency of each species of bacteria isolated by the use of the plate and, by combining these, he expressed the zymotic ammonifying efficiency of the soil, basing this on the idea that the ammonifying efficiency of a soil is proportionate to the number of organisms and their ammonifying power.

Remy (38) suggested a simpler method: 10-gm. portions of soil were added to 100 cc. of a 1-per-cent sterile peptone solution in flasks which were then incubated at 20°C. for 4 and 8 days; the culture was then filtered and ammonia determined in 25 cc. of the filtrate by distillation with magnesium oxide. The amount of ammonia formed served as an index of peptone decomposition. Four soils were tested by this method in May, July and October. By comparing the amounts of ammonia formed in peptone solutions in 4 days by 3 soils with the corresponding crop yields, he obtained the ratios of 100/100, 144/138 and 156/163. After 8 days' incubation, however, all the soils were found to give at all times about equal amounts of ammonia. Remy concluded from these results that peptone decomposition can serve as an index of the ability of a soil to decompose nitrogenous organic matter and, therefore, of soil fertility.

By comparing the amounts of soil used for the inoculation of the peptone solution, Löhnis (32) found that an average error of 2.84 per cent is involved when 10 per cent of soil are used, 8.35 per cent with 2 per cent of soil, and 12.30 per cent with 0.1 per cent of soil. In other words, the smaller the amount of soil used for inoculation, the greater is the error involved. As a result of a study of the decomposition of various peptones as well as other proteins during various periods of the year, Löhnis came to the conclusion that the soil does not possess a constant protein decomposing power and, for the study of the influence of treatment and environmental conditions, samples should be taken as often as possible throughout a whole period. Gutzzeit (17) used 1-per-cent peptone solution in soil extract and found two different soils to give the same amount of ammonia after a 5-day incubation period at 20°C. However, a distinct difference in the nitrifying capacity was obtained between the two soils. Buhler and Fickendey (6) used for inoculation of the peptone solution a suspension of soil in water rather than the soil itself and a temperature of incubation of 25°C. but no difference could be obtained in the ammonia-forming capacity of different soils. Lipman (23) compared the ammonifying capacity of four soils in peptone solution, using for inoculation both the soil itself and a soil suspension in water. Much higher results were obtained with the direct inoculation with soil at a temperature of 20°C. for 82 hours. However, when 0.5 per cent  $K_2HPO_4$ , 0.2 per cent  $MgSO_4$ , 0.2 per cent  $CaCl_2$  and sufficient sodium hydroxide to make the reaction weakly alkaline were added to the peptone solution, not only was there no difference between the two methods of inoculation, but there was no difference between the four different soils. It was suggested, therefore, that, for the study of the peptone-decomposing power of the soil, it is best to use only peptone in tap water. However, even if slight differences are obtained in favor of one soil over another when using only peptone in water, they may be due not so much to the fertility of the soil expressed by a more active bacterial flora as to the presence of larger quantities of soluble phosphates and other minerals in one soil than in another.

Vogel and Zeller (45), by incubating their cultures for 6 days at 20°, obtained no difference in the peptone-decomposing power of different soils taken at different times; if any differences were obtained, they were not greater than the error between duplicate determinations. Even slight differences in the temperature of incubation show greater differences than the physico-chemical properties of the soil itself. By replacing peptone by hornmeal greater differences were obtained due to the slower reaction.

Albert and Luther (1) found the Remy method to give valuable information on the chemico-biological processes in forest soils. Vogel (44) as well as Lemmermann and associates (21) found that various soils show little difference in ammonifying efficiency when inoculated into solutions; differences appear only when nitrification takes place. If any differences are obtained by carrying out the ammonification test according to Remy, they are chemical rather than bacteriological in nature. That the method of Remy has little justification has also been pointed out by Ritter (40) in the study of the influence of drying on the biological activities in the soil. Lipman (23, 24) found that the peptone-decomposing power of a soil is greatly influenced by the application of manure. Lipman attempted to establish a direct relationship between crop production and protein-decomposing power, using 1-per-cent

peptone, casein, albumin, but without any satisfactory results. Remy (38) replied that the decomposition of proteins by soil should not be looked upon as of deciding moment in soil fertility.

Fischer (12, 13) as well as Dzierbicki (10) pointed out that the chemical composition of the soil introduced with the soil infusion and particularly the content of phosphates has a greater influence upon ammonia formation in peptone solution than the bacterial population. Rahn (37) also suggested that differences obtained, by the solution method, between various soils, are due largely to the mineral content of the soil inoculum.

Löhnis (32, 33) was the first to modify the peptone solution by using, in place of water, an extract of soil, to which peptone or other protein material is added. Fischer (11) soon pointed out, however, that, under these conditions, the difference in ammonia formation may be due rather to the extract than to the inoculated soil.

The original Remy method was modified by Remy and Rösing (39) whereby 10 per cent of soil in the form of a soil suspension (50 gm. of soil in 250 cc. sterile physiological salt solution shaken for 5 minutes) is used for inoculation, cultures incubated for 4 days and ammonia determined in the whole culture including the soil. By using various proteins and peptones, Remy concludes that the peptone decomposition in solution is an index of the protein decomposition in the soil. In view of the fact that peptone is too rapidly decomposed in comparison with the nitrogen compounds in the soil, it is suggested to use 1 per cent of pure gelatin, 0.1 per cent each of  $K_2HPO_4$ ,  $MgSO_4$  and  $Na_2CO_3$  and 10 per cent of sterile soil extract; 0.1–0.25 gm. of soil in suspension should be used for the inoculation of 50 cc. of the cultures, which are then incubated at 20°C. for 7–8 days. Voitkevitch and Kolenev (46) obtained no greater differences between the peptone-decomposing power of five different soils than between duplicate checks of the same soil.

In view of the fact that the solution method was found unsatisfactory, Vogel (44, 45) suggested using the soil itself: adding 5 gm. of hornmeal to 500 gm. of sieved soil, mixing well, adding 12 per cent water after and placing in flasks, plugging with cotton and incubating 12 days at 23°C. By determining the organic, the total, the ammonia, and the nitrate nitrogen, Vogel demonstrated that the decomposition of hornmeal takes place in different soils with the same intensity, but the differences are found only when the ammonia is transformed into nitrates.

It is interesting to note that the predominance of certain groups of bacteria in laboratory tests using the solution method, depends upon the kind of protein used, as pointed out by Löhnis and Parr (36).

No relation between bacteriological activities in soil and in solution were found by Stevens and Withers (41, 42), the results being very variable, some bacterial soil complexes ammonifying faster in soils than in solutions and vice versa. They suggested standard methods for ammonification studies with soils, by determining the ammonifying efficiency, ammonifying inoculating power and ammonifying capacity, and believed that the results obtained by using soil as a medium were more trustworthy. Löhnis and Green (35) claimed that no necessary fundamental difference should be regarded as existing between bacteriological processes in soil and solution media.

Remy and Rösing (39) also suggested that more positive information is obtained in soil than in solution. Lipman and associates (25, 30) found, by the addition of dried blood or other nitrogenous organic matter to 100-gm. portions of soil, keeping at suitable moisture and temperature conditions for several days, that ammonia formation may serve as an index of the intensity of the decomposition processes in the soil. This method (tumbler method) has come into general use and is similar to determining the ammonifying efficiency suggested by Stevens and Withers. Lipman and Brown (29) obtained similar results by the solution and soil tests. However, the variability between the duplicate determinations and the difference in the amounts of ammonia formed from the different nitrogenous materials are of such a nature that one may question the value of the ammonification test altogether. We are particularly justified in doing that when we compare the results obtained by these

investigators from the ammonification tests, with the bacterial numbers and nitrifying capacity of the same soils. The latter two methods yield uniform results, while there is an entire lack of uniformity in the ammonification tests.

On studying the influence of seasonal variation upon ammonia formation in soil and in solution, Löhnis and Green (35) found this variation to lie on the border of experimental error between duplicate tests.

Greaves (15) found no difference in the ammonia formation of virgin and cultivated soils, although a nearly quantitative relationship was found to exist between the number of the colonies, nitrification and nitrogen fixation. In a latter study of the influence of manure on the soil, Greaves and Carter (16) found a direct relationship to exist between the bacterial numbers, ammonifying and nitrifying powers and crop production.

Brown (4, 5) found a close correlation between crop-producing and ammonifying power of the soil. Brown (3) states, in another place, that fresh soil is the most rational medium for studying the physiological activities of soil bacteria. Fresh soil permits of the greater differentiation in field soils differently treated, according to the activities of ammonifying bacteria, than air-dry soil inoculated with the soil infusion. This need not necessarily point to the value of the ammonification test, since we might conclude that it is the physical and chemical condition of the soil which account for the differences in ammonia formation rather than the bacteriological condition. Where the same air-dry soil is used for the inoculation with suspensions of various soils, we get little differentiation due to the fact that the medium has the same physical and chemical condition. This will also account for the fact that Brown found air-dry soils from particular plots inoculated with infusions of fresh soils to show greater differences than an air-dry standard soil inoculated with infusions from the various soils. Brown himself recognized that "the chemical character of the soil is of considerable moment when considering its ammonifying efficiency."

Kelley (18) found that both good and poor soils supported vigorous ammonification. Gainey (14) concludes from a study of various soil types, of different productivity, from widely different localities that there is no correlation between yield and ammonia nitrogen content. Lipman and Burgess (22) found the nitrification test a more reliable criterion than the test of ammonification. By comparing a large number of soils, they found a decided tendency toward a more or less uniform ammonifying efficiency of all the soils tested. They suggested the discarding of the ammonification method. Burgess (7) states, as a result of studies on Hawaiian soils that, as a rule, ammonification tests are not suitable in differentiating good and poor soils, although they often show differences between very poor and very good soils. A lack of correlation between ammonification studies and crop productivity has previously been pointed out by Fischer (13). Temple (43), as a result of extensive studies concludes that "The ammonification test is of little value in determining the nature of the soil micro-flora, since, (a) the soil medium largely controls the rate of ammonia production, (b) all soils are well supplied with bacteria, capable of changing proteid nitrogen to ammonia if the soil is made a suitable medium, and (c) the test as usually conducted does not take into consideration the considerable amounts of ammonia that may escape into the atmosphere or be converted to nitrate or nitrite. Ammonification can properly be used by the soil chemist as a test of soil fitness."

#### THEORETICAL

As seen from the brief historical review, we are still uncertain as to whether ammonification studies can be used for the interpretation of soil fertility. Some investigators (Fischer and Temple) condemn these studies as valueless in determining the nature of the soil flora. At best they are merely an indication of soil fitness, since the ammonia formation in the soil is largely controlled by the soil medium. Others (Brown) still continue to use ammonification

studies for measuring soil fertility and even attempt to predict fertility from these data. Before taking up the experimental results which will tend to clear up the subject in question, it may not be out of place to discuss first a few chemical and biological principles involved in ammonia formation from proteins in the soil or in solution inoculated with soil.

The process of ammonification is extremely complex, from the biochemical viewpoint, and is most intimately connected with the decomposition of protein materials in the soil, as pointed out by Lathrop (20). Then there are a large number of groups of microorganisms in the soil that are able to break down proteins with the formation of ammonia, some at a rapid speed, others more slowly, still others only very slowly, depending upon the nature of the organism, length of period of incubation, nature of culture medium and presence or absence of carbohydrates and other non-protein substances. A change in the physical and chemical condition of the soil will affect in a different way the activities of these different groups of soil microorganisms and the type of organic compounds formed from the decomposition of proteins. The majority of organisms developing on the common plate used for counting bacteria belong to one of four groups, viz., the spore-forming and non-spore-forming bacteria, fungi and actinomycetes, and all of these are able to form ammonia from proteins. The speed of the reaction, however, will be different for these four groups. The fungi break down proteins in the soil very rapidly and within 7-10 days 50 per cent of the nitrogen in the protein may be transformed into ammonia by pure cultures of these organisms. The actinomycetes will, in that period of time, due to their comparatively slow growth, produce only little ammonia; when, however, the analysis is made after 30-50 days, it will be found that the actinomycetes as a rule will have allowed as great an accumulation of nitrogen as the fungi if not greater. The same may be true of the two groups of bacteria. While the general impression has been that the spore-forming bacteria are the most active ammonia-forming organisms in the soil, due to their rapid development on the plate, Conn and Bright (9) found that a non-spore-forming organism will produce much more ammonia from manure than the spore formers.

Modifying the soil, as by adding organic matter, inorganic fertilizers, lime, moisture, cultivation, etc., does not modify the activities of these various groups of soil organisms to the same extent. The addition of lime, for example, to an acid soil results in a corresponding increase in the number of actinomycetes and decrease in fungi. It may result in slower, but more accumulative formation of ammonia, so that, in a brief period of incubation, the acid soil may produce more ammonia, due to the relative greater abundance of fungi, while the limed soil may give a higher ammonia formation when the period of incubation is prolonged. Then the lime treatment of an acid soil will make conditions more favorable for the activities of the nitrifying bacteria which will transform a part of the ammonia into nitrates. A number of other reactions may be brought about by the lime treatment which will affect the

amount of ammonia accumulated in the soil, when a comparatively large amount of protein has been added to the soil, under artificial laboratory conditions. To state that ammonia accumulated, under these conditions, is an index of soil fertility, with a good many results pointing the other way, is not scientifically correct.

Conditions which tend to bring about a change in the groupings of the four groups of microorganisms will also affect the amount of ammonia that will accumulate. When a small quantity of soil is introduced into a peptone solution, the rapidity of the reproduction of the various microorganisms, particularly bacteria, will bring about as rapid an increase in numbers in the flasks inoculated with a good fertile soil as in those inoculated with a poor soil. After all, the difference between the total numbers of microorganisms in a fertile and a corresponding unfertile soil may be about 3 to 1, yet it will not take very long before the flasks inoculated with the latter will contain as many bacteria as the flask inoculated with the former as pointed out by Rahn (37). This is true, of course, when the solution contains, in addition to the peptone, also the minerals necessary for the activities of the microorganisms. When however, only peptone (or any protein) solution is used, the introduction of the necessary amount of soluble minerals with the amount of soil used for inoculum will affect the rapidity of multiplication of the bacteria and, therefore, the ammonia formation. In this case we are testing not the bacterial activities in the soil, but the presence of soluble minerals, which can be accomplished much more readily by direct chemical analysis. This was recognized by Löhnis (33, 34) and Remy (39) who suggested the addition of soil extract to the culture.

Another disadvantage of the solution method consists in the fact that two of the most active protein-decomposing organisms in the soil the fungi and actinomycetes, do not have a chance to develop in the solution due to the rapid development of bacteria, rapid accumulation of ammonia and short period of incubation. Only the aerobic and the facultative anaerobic, the so-called putrefactive bacteria, develop predominantly and these, by their rapidity of multiplication, universal distribution, will readily account for the lack of differentiation of soil bacteriological activities, by the solution method. If there is any difference at all, it can readily be accounted by the difference in the chemical composition of the inoculum.

Ammonia is formed by the various groups of soil microorganisms chiefly as a waste product in the utilization of proteins as a source of energy. It is also produced, of course, in the nitrogen metabolism of the organisms, in the hydrolysis of proteins and their degradation products by enzymes and in the autolysis of the microbial cells, but to a comparatively much smaller extent. Microorganisms can use both carbohydrates and proteins as sources of energy. When there is present in the soil, in addition to proteins, a sufficient supply of carbohydrates, the microorganisms will use the latter as a source of energy and will attack the proteins only in so far as they need nitrogen for their

metabolism, in this case very little ammonia will accumulate in the soil and, even if some is produced, it may rapidly be used up by various other microorganisms as a source of energy. This will account for the small amount of ammonia formed or accumulated in the soil, when dextrose or other available carbohydrates and even straw are added to the soil, as shown by Lipman and associates (28), Waksman (48) and others. But, when there is an insufficient amount of available carbohydrates in the soil, the microorganisms will attack the proteins not only as a source of nitrogen, but as a source of carbon, which yields the necessary energy. The proteins contain about 50 per cent of carbon and about 10 to 15 per cent of nitrogen. The microorganisms require a great deal more carbon than nitrogen for their metabolism. The carbon will then be rapidly oxidized, while the excess of nitrogen will be left in the medium as a waste product, in the form of ammonia.

According to Kruse (19) the weight of the fungus body will be, under the most optimum conditions, about one-third of the sugar consumed. Protein or protein-degradation products are used much less economically, so that, for 1.5 gm. of peptone consumed there is a yield of about 0.162 gm. of fungous mycelium which will contain about 12-15 mgm. of nitrogen. One and one-half gram of peptone may contain about 200 mgm. of nitrogen, so that 90 per cent of the nitrogen will be left as a waste product in the form of ammonia, with peptone as the only source of energy. The utilization of available energy by bacteria is even less efficient. *B. proteus*, for example, consumed, in a period of 30 days, out of 793 mgm. available nitrogen only 97 mgm., when it had to derive its energy from proteins. Urea bacteria, according to Kruse (citing the work of Miquel), convert 4000 parts of urea to build up 1 part of body substance. The addition of a small amount of available carbohydrate will check for a short period of time the ammonia accumulation, although it will result in an increased microbial flora. After all the carbohydrate is used up, the microorganisms will attack the proteins present and may even produce larger quantities of ammonia in the soil than when no carbohydrate has been added, as pointed out by the author elsewhere (47). However, when the amount of carbohydrate added is large, it will prevent the ammonia accumulation for some time and perhaps for the whole length of the usually short period under which the laboratory tests are carried out.

#### EXPERIMENTAL

The soils used in the following investigations were obtained from a series of plots laid out by this department 15 years ago and receiving the same fertilizer treatment. A careful record of treatments and crop yields was kept and has been discussed by Lipman and Blair (26) and by the author (48). These plots have been so modified by the continuous treatment as to establish definite microbiological differences in the study of numbers of microorganisms, as pointed out in the previous paper (48) and as will be shown in the following paper in the study of the nitrifying capacity of these plots.

The plots seem to be, therefore, well suited for finding out whether ammonification studies by one or all the methods in use can indicate differences in soil fertility. The treatment of the plots, carbon and nitrogen content at 1917 and total crop yield for the last 14 years are reported in table 7. Both the solution methods and soil methods were employed, the latter in connection with the study of nitrification, soil reaction and numbers of microorganisms.

#### AMMONIA FORMATION IN SOLUTION

The solution method was carried out as follows: 100 cc. portions of 1-per cent solutions of Bacto-peptone, casein or Gold Label gelatin in distilled water with or without 0.05 per cent  $K_2HPO_4$ , were placed in 250-cc. Erlenmeyer flasks, plugged with cotton and sterilized, for 15 minutes at 15 lbs. pressure. The casein was previously dissolved in 0.1 *N* NaOH, then adjusted with 0.1 *N* HCL to a reaction of pH 6.5-7.0. The flasks were inoculated with 10 cc. of a 10-per-cent suspension of the soil shaken for 5 minutes, unless otherwise stated, and incubated at 28-30°C. for 3-7 days. The whole culture was then transferred into copper flasks and the ammonia determined by distillation with MgO, in the usual manner. Two to four flasks were used for each determination, but only the averages are given, in view of the fact that the duplicate determinations checked up fairly well in the majority of cases.

The results presented in table 1 point out clearly the fact that the amount of inoculum plays an important part, when only a 1-per cent peptone solution is used. The influence of inoculum was studied in the presence of 0.05 per cent  $K_2HPO_4$  in order to find out whether the differences in ammonia formation are due to the specific microbiological flora of the plots or to the larger supply of soluble phosphates and potassium salts in the more fertile plots than in the less fertile plots. For this purpose only 5A and 7A were used as representing the most productive and least productive plots, without the interference of the lime factor.

Table 2 shows that much smaller differences are found between the fertile and non-fertile plot, when  $K_2HPO_4$  is used. Although in most cases the soil from the fertile plot (5A) gave higher ammonia formation than the soil from the unfertile plot (7A), the reverse held true in one case.

No period seems to show any advantage over any other in bringing out differences in ammonia formation important enough for differentiating the more fertile from the less fertile plot, although in all cases the amounts of ammonia formed by the former was somewhat higher than those formed by the latter.

A series of plots were next studied for the influences of fertility upon ammonia formation by the respective soils in solution of various proteins. Table 3 shows that the limed plots had in all cases a slightly higher ammonia-content than the non-limed plots; practically no correlation is found between the fertility of the plots and ammonia formation in solution. Plots 5A and 5B are the most fertile, 7A and 11A had practically no crops due to the excessive



acidity, 7B and 11B were intermediate. Plot 11A which is so acid as not to allow any crop growth, gave in the peptone solutions a greater amount of ammonia than 5A; 7B which received nothing but lime for the last 15 years gave, both in the peptone and casein solutions, higher amounts of ammonia than 5B, the heavily manured plot.

TABLE 1

*The influence of amount of inoculum upon ammonia formation in 100 cc. of a 1-per-cent peptone solution\**

PLOT NUMBER	NH <sub>3</sub> -N FORMED WITH VARIOUS AMOUNTS OF INOCULUM		
	0.01 gm. of soil	0.1 gm. of soil	1 gm. of soil
	mgm.	mgm.	mgm.
5A	42.28	46.78	55.72
5B	40.32	48.02	55.58
7A	30.80	41.86	44.94
7B	29.96	40.04	43.82
11A	32.34	41.30	49.56
11B	35.15	45.64	52.08

\* Cultures incubated 3 days. Soil sampled January 28, 1922.

TABLE 2

*The influence of amount of inoculum and period of incubation upon ammonia formation, in 100 cc. of 1-per-cent peptone solution with 0.05 per cent of K<sub>2</sub>HPO<sub>4</sub>*

PROTEIN SOURCE	PERIOD OF INCUBATION	AMOUNT OF INOCULUM	NH <sub>3</sub> -N FORMATION	
			5A	7A
	days	gm.	mgm.	mgm.
1-per-cent peptone solution .....	1	1.0	11.25	10.28
	2	1.0	55.14	51.27
	3	0.1	76.16	70.49
	3	1.0	78.87	76.53
	3	10.0	93.94	77.07
	7	1.0	103.18	100.38
1-per-cent casein solution .....	7	0.1	94.50	89.18
	7	1.0	99.96	93.94
	7	10.0	89.04	95.76

The same experiment was repeated with the soil from 4 distinct plots 5A, 7A, 5B, 7B, using small and large amounts of inoculum, in the presence and absence of K<sub>2</sub>HPO<sub>4</sub>.

It is interesting to note (table 4) that in all cases there is an increase in ammonia formation with an increase in the amount of inoculum used. Plot 5A, which is a very fertile soil, gave in the absence of phosphates higher amounts of ammonia than 7A which lacks in fertility; in the presence of phosphate, the differences are very small, particularly with the larger amounts

of inoculum. Plot 5B, however, which received the same treatment as 5A in addition to lime, gave lower amounts of ammonia both with small and large inoculum, in the presence and in the absence of phosphates.

The results obtained from the above experiment would lead us to condemn completely the study of ammonification in solution and any attempts to correlate soil fertility with any differences in ammonia produced as fultile.

TABLE 3  
Formation of ammonia in 100-cc. portions of 1-per-cent solutions of various nitrogenous substances\*

PLOT NUMBER	PEPTONE		CASEIN		GELATIN
	No phosphate†	0.05 per cent $K_2HPO_4$ ‡	No phosphate†	0.05 per cent $K_2HPO_4$ ‡	No phosphate†
	mgm. $NH_3-N$	mgm. $NH_3-N$	mgm. $NH_3-N$	mgm. $NH_3-N$	mgm. $NH_3-N$
5A	78.87	82.7	77.9	89.96	47.1
5B	83.02	83.9	78.3	89.54	58.1
7A	76.53	84.2	78.8	83.94	42.5
7B	88.5	89.26	82.4	90.10	47.7
9A				86.60	
11A	81.29	88.5	77.7	84.50	41.2
11B	87.69	88.7	78.7	84.64	42.4
19A	82.67			85.34	42.4

\* One gram of soil (5 cc. of a 20-per-cent suspension) was used for inoculation.

† The reaction of the protein solutions was adjusted to pH = 7.0. Peptone cultures incubated for 4 days, casein and gelatin for 6 days. 10 cc. of 10-per-cent soil suspension was used for inoculation.

‡ The cultures with the phosphate were prepared a week after the casein cultures without the phosphate.

TABLE 4  
Ammonia formation in 100 cc. portions of solutions as influenced by amount of inoculum and presence of  $K_2HPO_4$

PLOT NUMBER	1-PER-CENT PEPTONE			1-PER-CENT PEPTONE AND 0.5 PER CENT $K_2HPO_4$		
	0.01 gm. soil	0.1 gm. soil	2 gm. soil	0.01 gm. soil	0.1 gm. soil	2 gm. soil
	mgm. $NH_3-N$	mgm. $NH_3-N$	mgm. $NH_3-N$	mgm. $NH_3-N$	mgm. $NH_3-N$	mgm. $NH_3-N$
5A	80.08	80.08	87.64	76.16	79.38	86.80
7A	67.76	76.72	83.02	71.40	78.12	84.84
5B		78.12	85.96		78.96	84.28
7B		81.62	88.76		85.12	88.62

#### AMMONIA FORMATION IN THE SOIL

The extensive amount of work done on the formation of ammonia, by the use of fresh soil, and the claims put forth (Brown) that these results are parallel with crop production might lead one to look here for a method differentiating soils of different fertility. For these experiments the fresh soil

was placed, as soon after sampling as possible, in 100-gm. portions into tumblers. One-gram portions of dried blood (11.50 per cent nitrogen) were well mixed with the soil. The tumblers were covered with glass and placed in the incubator, 28–30°C. for 7 days. The moisture was kept up to optimum by addition of distilled water. The ammonia was then determined by the usual method. In some cases, 200-gm. portions of soil with 2 gm. of dried blood were placed in the tumblers, and, in addition to ammonia determinations, pH and nitrate determinations were made. Duplicates or triplicates were always made, but only averages are recorded, since duplicates usually agreed well.

The results presented in table 5 were obtained at three different periods when samplings of soil were made, January 28, April 11 and May 22, 1922. In the case of the winter sampling, the tumblers were allowed to remain for one or two days uncovered, till the moisture was reduced to the optimum.

TABLE 5  
*Ammonia formation in 100 gm. fresh soil*

FLOT NUMBER	JANUARY 28	APRIL 11	MAY 22
	<i>mgm. NH<sub>3</sub>-N</i>	<i>mgm. NH<sub>3</sub>-N</i>	<i>mgm. NH<sub>3</sub>-N</i>
5A	30.87	50.7	45.22
7A	32.76	38.1	38.08
9A	.....	....	34.02
11A	38.50	44.1	44.24
19A	.....	....	39.34
5B	18.41	41.9	42.00
7B	15.26	45.2	40.04
11B	28.49	56.7	47.46

The results presented in table 5 indicate that not only is the use of ammonia formation in the soil as an index of soil fertility unjustified but that there is no soil differentiation at all. The reason for the low results in the January test is probably the insufficient aeration of the soil which was placed in the tumblers in a saturated condition.

The results become more interesting when we compare the ammonia formation at different periods. Table 6 shows that ammonia accumulation in the soil was higher in the acid and less fertile plots. This is due not so much to their greater capacity of forming ammonia as to their lower capacity of transforming the ammonia into nitrates, which is an entirely different story. This question will be discussed at length in the following paper of this series.

It becomes at once apparent, from table 6 that the amount of ammonia accumulated in the soil, after a certain period of time, is not at all an index of the fertility of the soil, but may merely indicate how incapable a certain soil is of transforming that nitrogen into nitrates.

The results here reported represent only a few experiments selected at random from a large number of investigations on the relation of ammonia for-

TABLE 6

Course of ammonia accumulation and nitrate formation in 100 gm. of soil and 1 per cent of dried blood

PLOT NUMBER	NH <sub>3</sub> -N AFTER				NO <sub>3</sub> -N AFTER 27 DAYS	TOTAL NH <sub>3</sub> AND NO <sub>3</sub> -N
	3 days	7 days	15 days	27 days		
	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
5A	11.69	45.22	77.56	69.80	42.2	112.00
7A	11.90	38.08	63.70	72.50	4.8	77.30
9A	9.38	34.02	70.42	78.96	7.6	86.56
11A	14.56	44.24	76.16	90.30	5.0	95.80
19A	11.34	39.34	74.06	88.20	8.8	97.00
5B	13.16	42.00	58.10	49.70	43.2	92.90
7B	12.04	40.04	53.20	57.80	5.4	63.20
11B	10.50	47.46	59.78	69.20	4.9	74.10

TABLE 7

Biological analysis of soil as an index of soil fertility

PLOT NUMBER	NH <sub>3</sub> -N* PEPTONE SOLUTION	NH <sub>3</sub> -N† CASEIN SOLUTION	NH <sub>3</sub> -N‡ IN SOIL	NO <sub>3</sub> -N§ IN SOIL, D.B.	NO <sub>3</sub> -N§ IN SOIL (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	BACTERIAL NUMBERS	CROP YIELDS PER ACRE FOR 14 YEARS	REACTION	SOIL TREATMENT¶	TOTAL CARBON	TOTAL NITROGEN
	mgm.	mgm.	mgm.	mgm.	mgm.	mil-lions‡	lbs.	pH		per cent	per cent
5A	78.87	99.96	45.22	42.2	4.30	17.84	60,541	5.4	Minerals, 16 tons of cow manure	1.440	1.185
7A	76.53	93.94	38.08	4.8	0.56	7.14	15,295	4.6	No fertilizer	0.930	0.785
9A	87.41	96.60	34.02	7.6	4.80	18.25	50,488	5.9	320 lbs. NaNO <sub>3</sub> , minerals	1.130	0.975
11A	81.29	94.50	44.24	5.0	0.51	4.80	38,731	4.4	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> equivalent to 320 lbs. NaNO <sub>3</sub> , minerals	1.210	0.904
19A	82.67	95.34	39.34	8.8	1.56	8.20	27,227	5.5	Minerals only	1.010	0.872
5B	83.02	99.54	42.00	43.2	12.40	13.40	55,034	6.4	Manure, minerals, lime	1.420	1.143
7B	89.26	100.10	40.04	5.4	7.20	11.70	27,239	6.3	Lime only	1.020	0.821
11B	87.67	94.64	47.46	4.9	3.80	12.74	53,826	5.7	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> , minerals, lime	0.060	0.819

\* Peptone cultures (10 cc. of 10-per-cent suspension of soil in water added to 100 cc. of 1-per-cent peptone and 0.05-per-cent K<sub>2</sub>HPO<sub>4</sub> solution) incubated for 3 days.

† Casein cultures (same medium) incubated 7 days.

‡ NH<sub>3</sub>-N in soil signifies ammonia formed in 7 days from 1 gm. of dried blood in 100 gm. of soil.

§ NO<sub>3</sub>-N signifies nitrate nitrogen formed in 100 gm. of soil from 1 gm. of dried blood (D. B.) or from 30 mgm. of N as ammonium sulfate, in 30 days.

¶ Fertilizer quantities and yields are given on acre basis. The B plots received 1 ton of ground limestone per acre in 1908, 2 tons in 1913, and 2 tons in 1918.

¶ Numbers show total number of microorganisms developing in 7 days, at 28-30°, on albumen agar plate, in millions per gram of soil.

\*\* For this apparent lack of correlation see discussion on p. 62 of text.

mation to soil conditions and crop productivity. Results have been negative, with very few indications of differentiation. This is particularly striking in view of the fact that a definite correlation is found between soil productivity and numbers of microorganisms as shown previously and the nitrifying capacity of the soil, as will be shown in a later paper. A complete summary of results obtained on the ammonification on May 22 as correlated with some other soil biological phenomena and crop yields are given in table 7.

There is, a definite correlation between numbers of microorganisms and crop yields, also between these and nitrifying capacity for the unlimed plots only. The bacterial numbers for plot 9A run lower on the average than those of 5A as shown from the yearly average given by the author (48, p. 338). The large differences between the nitrification of dried blood and ammonium sulfate is limed and unlimed soils and apparent lack of correlation with bacterial numbers and crop yields is due entirely to the inadequacy of the methods used as present for measuring nitrification, as will be shown in the following paper. The complete lack of correlation between ammonification in soil and in solution and crop productivity bears out the statement previously made as to the unreliability of this method as a soil bacteriological function.

The results obtained from measuring ammonia accumulation in the soil, which is only a product resulting from the artificial development of a heterogeneous group of organisms some of which may or may not be inhabitants of the particular soil, do not correlate with soil productivity and other soil bacteriological activities as indicated by number of microorganisms, nitrifying capacity, carbon dioxide-producing capacity, etc. We may still, however, be able to obtain from the rapidity of decomposition of proteins as indicated by the disappearance of the proteins from the soil, either as a result of the activities of the microorganisms themselves or their enzymes, a reliable index of soil fertility.

#### SUMMARY

1. Ammonification in solution cannot be used for soil differentiation, for the following reasons:

a. Of the three large groups of soil microorganisms, only the bacteria develop in solution, while the fungi and actinomycetes do not usually develop because of the nature of the medium and period of incubation.

b. Of the bacteria, those which develop are only the so-called putrefactive forms or those that are able to grow rapidly in liquid media, with peptone or other protein or protein derivative, as the only source of carbon and nitrogen. These forms are usually present in all soils, independent of the fertility of the particular soil.

c. Even where differentiation between soils is obtained it may be due more to the presence or absence of certain minerals in the soil introduced as an inoculum rather than to the particular soil flora.

2. Actually no definite correlation was found between ammonia formation in solution on the one hand and crop production, bacterial numbers and nitrification on the other.

3. Ammonification in soil cannot be used as an index of soil fertility:

a. All soils contain various groups of microorganisms that are able to break down complex proteins, with the formation of ammonia.

b. The final amount of ammonia accumulated in the soil is a resultant of a number of factors, among which are the nature of the protein used in carrying out the test, the presence or absence of available carbohydrates, the initial reaction of the soil and buffer content of the soil, the rapidity with which the ammonia is transformed into nitrates, which depends upon the initial reaction of the soil, buffer content, etc., and, the loss of ammonia into the atmosphere.

4. Ammonification can be used only in comparing the activities of specific cultures of microorganisms under controlled conditions and in studying the course of rapidity of decomposition of organic matter. It cannot be used in determining the nature of soil fertility or soil micro-flora.

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ON THE EIGHTIETH ANNIVERSARY OF THE BIRTH OF  
DR. PAUL WAGNER

TRANSLATED BY THE COURTESY OF  
H. G. HUSTON

*Potash Syndicate*

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On March 7 the noted German agricultural chemist and investigator, Dr. Paul Wagner of Darmstadt, will complete his eightieth year, at which time his numerous friends and pupils in Germany will hold a celebration at Darmstadt in his honor.

Fifty years ago, Doctor Wagner became Director of the Experiment Station at Darmstadt, which had just been founded, and has since won for this institution a world renown, through his investigations on plant foods. Doctor Wagner certainly deserves great commendation for having, with the help of his own method of pot experiments, substantially extended, and firmly established the foundation for the use of commercial fertilizers.

He was the first to recognize and correctly estimate the fertilizing effect of Thomas Phosphate or basic slag.

By a steady improvement in the methods of fertilizer experiments in the field, he succeeded in making of these field experiments a practical means of exact investigation.

Doctor Wagner, furthermore, has clearly shown to the practical farmer the results of his investigations in the vegetation house, field, and laboratory. He has done this in articles which are easily understood and in inspiring lectures and in this way has contributed in an enormous degree toward the proper use of commercial fertilizers in agriculture.

Here in the United States, many of Doctor Wagner's articles are known and have been translated or summarized by numerous writers to the great advantage of American agriculture.



## FURTHER NOTES ON THE GROWING OF WHEAT IN ONE-SALT SOLUTIONS

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By employing certain combinations of one-salt nutrient solutions and systematically changing the cultures growing therein from one solution to another, the writer (1) has shown that wheat seedlings grow about as well, for at least one month<sup>1</sup> with only two nutrient elements (one salt) present in the media at a time, as they do in complete, well-balanced, nutrient solutions. It was held that these results showed that the proper pairing of certain of the nutrient cations with anions operated as a very important physiological condition in the growth media of the plants. The term "proper pairing" of the ions is not meant to convey the idea that the intake of nutrient mineral elements by the plant was conditioned by the law of definite chemical proportions, either for the absorption of two ions of opposite charge or any other group of atoms. But the conclusion drawn from the results of the experiment was that there are elements whose availability to and utilization by the plants is dependent upon certain other elements in the medium. These ions being of opposite charge are, therefore, considered as being physiologically paired. This proper pairing of the ions, therefore, would designate a salt which, when used singly, would give greater growth of plant than any other nutrient salt used singly that contains but one of these so-called properly paired elements. If these conclusions are stated in a concrete way, they are as follows:

The absorption and utilization of nitrates by the wheat plant is largely dependent upon the relative supply of available potassium in the medium. If either potassium or nitrate is to be utilized, both must be present and absorbed at the same time. The utilization of these elements by the plants, on the other hand, is largely independent of the presence at the same time, of other essential ions, namely, Ca, Mg,  $\text{PO}_4$  and  $\text{SO}_4$ , and perhaps Fe, though the effect of the absence of this latter element has not been experimentally determined. That

<sup>1</sup> Judging from an experiment now in progress, it appears to be possible to grow wheat plants to maturity by the method referred to in the paper cited, that are equally as good as those grown in any of the complete, well-balanced, nutrient solutions tested by the writer. Plants now ten weeks old growing in solutions of  $\text{KNO}_3$ ,  $\text{CaSO}_4$  and  $\text{MgHPO}_4$ , and changed systematically from one solution to another, are fully as large as those growing simultaneously in the control complete nutrient solutions. The set of cultures giving best growth to date is one where the plants are in a solution of  $\text{KNO}_3$ , 4 days;  $\text{CaSO}_4$ , 1 day; and  $\text{MgHPO}_4$ , 1 day. These different solutions are each of 0.01 mol. concentration.

is, these other elements are not needed to make potassium or nitrate physiologically available. Obviously, all essential elements must be accessible to the plant at times and at frequent enough intervals. They are important and needed for the growth of the plants, and this growth of the plant, undoubtedly, is in itself, a very important factor that affects not only the absorption and utilization of potassium and nitrate, but also that of all other needed elements.

Following up the investigation already referred to, which bore on the physiological importance of the relations between certain of the essential elements, data obtained from some recent simple experiments appear to throw further light on this important condition affecting the growth of wheat plants.

Cultures of wheat seedlings were set out in 1-quart jars filled with tap water which had a total salt concentration equal approximately to 0.1 atmosphere osmotic pressure. The elements that constituted the principal salts of the tap water were Ca, Mg, Na,  $\text{SO}_4$ , Cl. The plants remained in this medium for four months. The water was not renewed, save to replenish that lost by transpiration of the plants. During the first two months in tap water, the plants made some shoot growth attaining to a height of 14–20 cm. During this time, the plants made a very large root growth (2). The root mass attained at the end of this period varied from 90 to 120 cm. in length for different cultures. Approximately 50 per cent of the dry weight of the plants was contained in the roots. During the last two months that the cultures were in tap water, there was no apparent evidence of new growth of tops. As to the roots, it may be stated that growth appeared to have ceased three or four weeks before the end of the four months period. These plants of pale green and starved appearance which made no aerial growth for at least two months, but nevertheless possessed the ability to renew growth if placed in a good complete nutrient solution, were then transferred to the single salt solutions to be tested. One-quart containers were used and ten cultures were used for each test of the different salts. There was no further change of solution during the test of the remaining growth period of the plants, except the replenishment of water lost by transpiration. A trace of iron ( $\frac{1}{2}$  cc. of 0.01 mol.  $\text{FeSO}_4$ ) was added at intervals to the cultures. Some of the results obtained are given in table 1.

Ten days after the cultures had been transferred to the different salt solutions, it was noted that the plants placed in solutions containing nitrogen were greener than those placed in solutions that did not contain nitrogen. Within two to three weeks, new growth in the form of tillers began to appear at the base of plants placed in the solutions of  $\text{KNO}_3$ , the solutions of  $\text{Mg}(\text{NO}_3)_2$  and in the complete nutrient solutions, the latter being employed as controls. The tillers that started on the plants growing in  $\text{KNO}_3$  solution and the complete nutrient solution appeared to be normal. They grew and gained in height. But the tillers that started on the plants placed in  $\text{Mg}(\text{NO}_3)_2$  grew only to a height of 1–2 cm., then, due to abscission of the tips of the leaves and shoots, which is a characteristic phenomenon of cereal seedlings growing in certain unbalanced solutions, they could not elongate. A whorl of small,

deformed leaves, some of which died before the plants had lost their green color, was all the evidence of new growth obtained due to  $\text{Mg}(\text{NO}_3)_2$ . The plants of this set were the first ones to die. There was no evidence of new growth of the aerial portions of the plants placed in any of the solutions that did not contain nitrates. On the other hand, new roots started on the plants placed in the following solutions,  $\text{KNO}_3$ ,  $\text{CaSO}_4$ ,  $\text{CaHPO}_4$  and  $\text{Ca}(\text{H}_2\text{PO}_4)_2$ ,  $\text{MgHPO}_4$  and  $\text{Mg}(\text{NO}_3)_2$ . The new roots that formed on the plants placed in  $\text{Mg}(\text{NO}_3)_2$  soon died.

TABLE 1

*Some effects of single salt nutrient solutions on the renewal of growth of wheat seedlings\**

SALTS USED	CONCENTRATION OF SOLUTION	GROWTH RESPONSES	
		Tops	Roots
	<i>mol.</i>		
$\text{KH}_2\text{PO}_4$	0.010	No new growth	Little growth
$\text{KNO}_3$	0.010	Greening of leaves, formation of tillers, elongation of original stalks and tillers	New growth arising from root crown
$\text{K}_2\text{SO}_4$	0.010	No new growth	No apparent growth
$\text{CaHPO}_4$	Saturated		
$\text{CaH}_2\text{PO}_4$	0.001	No new growth	Some new growth
$\text{Ca}(\text{NO}_3)_2$	0.010	Greening of leaves, no new tillers	Some new growth
$\text{CaSO}_4$	0.010	No new growth	Little growth
$\text{MgHPO}_4$	0.010	No new growth	Very small amount of new growth
$\text{Mg}(\text{NO}_3)_2$	0.010	Greening of leaves, formation of new tillers which did not grow more than 2 cm. because of abscission caused by salt—plants were the first of experiment to die	A few new roots appeared but they soon died
$\text{MgSO}_4$	0.010	No new growth	No apparent growth
$\text{KH}_2\text{PO}_4$			
† $\text{Ca}(\text{NO}_3)_2$	0.010	Greening of leaves and much new growth of tillers and stalks	Much new growth
$\text{MgSO}_4$			

\* By renewal of growth is meant greening of the leaves and formation of new leaves or tillers.

† This was a complete nutrient solution composed of equal concentration of the three salts.

Of the nine different single salt-solutions used as growth media for wheat seedlings, only those plants in the solution of  $\text{KNO}_3$  produced appreciable growth. It was not as large as that produced by the plants placed in the complete nutrient solution, but, nevertheless resulted in significant increase of dry matter. As the tap water of the laboratory in which these plants were kept for four months contained appreciable amounts of Ca, Mg and  $\text{SO}_4$ , it appears that the plants may have absorbed certain amounts of these elements which possibly could have been used in a later growth phase. Potassium and

nitrogen were notably deficient in the tap water. It was only when these two elements were later contained in the growth medium of the plants that fairly good growth was obtained from wheat seedlings which had ceased growing for two months.

It is fully appreciated that wheat plants cannot grow in the absence of either potassium or nitrogen. That they did renew their growth in media where these two elements were present does not necessarily, by itself, imply a potassium-nitrogen relation as a physiological requirement for growth. But, in view of the findings of the experiment already referred to where it was only under those conditions where potassium and nitrogen were supplied together, other things being equal, that good growth was obtained and very poor growth when the two elements were not supplied together, the results here reported appear to be the only logical ones that could be expected to occur.

The reasons for this can be briefly stated:

(a)  $\text{KNO}_3$  supplied the wheat plant with two essential elements that are obtained by the plant through its roots. Apparently these two are needed in a greater quantity than any of the other mineral elements.

(b)  $\text{KNO}_3$  supplied the wheat plant with two elements that must be present at the same time in the growth medium in order that either of these essential elements can be utilized by the plants.

The results of these experiments with single salt solutions appear to have defined, at least in part, the basis that underlies the conditions for physiological balance between potassium and nitrogen in the culture solution of wheat plants. But the fact that  $\text{KNO}_3$  is the salt of potassium and of nitrogen which can be used singly to produce as good growth of wheat as was produced in the solutions of the six different types of three salt combinations used (1), does not mean that in order to obtain the best growth of wheat in a complete nutrient solution that potassium and nitrate must be present in that solution in the ratio that these elements are found in  $\text{KNO}_3$ . Neither does it mean that  $\text{KNO}_3$  is the best salt of potassium or of nitrogen to use to obtain the best growth of wheat plants in complete nutrient solutions. Experiments have shown that differences of ratios of ions in nutrient solutions may vary over a considerable range of values without any apparent correlatable differences in resulting growth of plant. This conclusion is further substantiated by the fact that the ratios of the essential mineral elements for plant growth as determined by the analysis of the water-soluble constituents of apparently equally productive soils cover a comparatively wide range of values. These facts, of course, in no way militate against the validity of the argument that the relations of the quantities of one element to that of another or others are physiologically important. It simply means that there appears to be no definite single ratio of a stated value of element to element that defines the one best set of conditions for the growth of plants in complex media. That there should be one best set of conditions for the growth of plants can hardly be expected in

the light of what is known of the effects of the interrelations of the factors (essential and non-essential) that influence plant growth. There appears to be a great array of different conditions under which plants do equally as well. This apparently must come as the result of the operation of Le Chatelier's law which appears to be equally applicable to biological processes as to chemical and physical, that is, that any alteration in the factors which determine an equilibrium causes the equilibrium to become displaced in such a way as to oppose, as far as possible, the effect of the alteration.

It appears that the physiological effect or the fertilizing efficiency of nitrogen is not primarily conditioned by the available supply of that element, but is affected in no small degree by the relation of the supply of that element to that of another, namely, potassium. The relation between these determines in no small measure whether one of the elements is deficient or not. Obviously the magnitude of available supply of any of the necessary elements in the growth media of plants is important because a certain minimum amount is required for normal metabolic processes of the plants, and because this factor (magnitude of supply) determines the relation of the actual mass of the one to that of any other.

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## RELATIONS BETWEEN CALCIUM CARBONATE, CERTAIN FERTILIZER CHEMICALS AND THE SOIL SOLUTION<sup>1</sup>

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### SOLUBILITY OF CALCIUM CARBONATE IN SOIL SOLUTION

The application by Johnston (9), of the fundamental laws of solutions to the relations between calcium carbonate in a solution and carbon dioxide in the atmosphere above it, has led the writer to study the relation of those laws to the use of lime in agriculture.

Lime is applied to the land in the forms of calcium carbonate, calcium hydrate or calcium oxide, depending on the economic conditions that surround its purchase and transportation. Its relatively low initial cost results in its application in large quantities per acre compared with most chemical fertilizers. It has been shown by MacIntyre (14) that the final stable form of lime in the soil is calcium carbonate, therefore the soil-solution under the conditions of customary applications of lime must be saturated with that compound for a considerable period.

Carbon dioxide is the important variable in the soil-solution because the range of its proportion in the soil-air is very wide in comparison with that in the free air above the soil. Air in the open country is remarkably uniform in its content of carbon dioxide; but in the soil, where the air must remain more or less stagnant, the respiration of the living organisms may cause the carbon dioxide to rise to high proportions as in ill ventilated rooms.

Carbon dioxide in the soil has received considerable attention by investigators and has been found to vary with soil and with crop. Russell (18) recapitulates such investigations and shows a range from 0.4 per cent to 2.3 per cent in pasture land, while as low as 0.05 per cent has been observed in bare sandy soil.

Turpin (24), in some vegetation experiments with pots, found a maximum of 3.34 per cent  $\text{CO}_2$  in the air of a clay soil on which a crop of oats was growing. Potter and Snyder (17) working on field soils found the  $\text{CO}_2$  to vary ten-fold in a few days on some of the plots studied. The writer has not attempted a comprehensive review of the studies of carbon dioxide in the soil-air, but only enough to show its probable range and consequent relations to the soil-solution and the calcium carbonate dissolved in it.

<sup>1</sup> Published by permission of the Director of the Massachusetts Agricultural Experiment Station.

Changes in soil-temperatures will vary the proportion of  $\text{CO}_2$  present, because the respiration of plants varies with the temperature in accordance with the principle of acceleration of chemical action by heat.

The  $\text{CO}_2$  in the soil-solution will be quickly affected by the changes in the soil-atmosphere for Johnston (10) states that electrical conductance shows the establishment, within ten minutes, of equilibrium between the  $\text{CO}_2$  of the air and an unstirred solution in an open beaker.

The concentration of carbon dioxide in the soil-solution varies with the proportion in the atmosphere, the temperature of the soil and the amount of salts in the solution. The coefficient of solubility decreases as the temperature rises and as the salt content increases; but in accordance with Henry's Law it remains constant when variations occur in the pressure of the gas above the solution. Solubility data for carbon dioxide in water and in salt-solutions have been determined by Just (12), Geffcken (6) and others, and their results have been compiled by Seidell (20) and by Cameron and Bell (2).

The mathematical relations between the carbon dioxide of the atmosphere and the calcium carbonate in the solution that is in contact with it, have been clearly discussed by Johnston (9) and also by Stieglitz (23). Several equations are required for their expression.

The solubility of  $\text{CO}_2$  is usually expressed in terms of liters of the gas, reduced to  $0^\circ\text{C}$ . and 760 mm., dissolved in one liter of the solvent. Since a gram-molecule of a gas normally occupies 22.4 liters, the solubility factor for  $\text{CO}_2$  at a given temperature divided by 22.4 gives the molar concentration of the solution at that temperature. This constant,  $c$ , multiplied by the partial pressure,  $P$ , of  $\text{CO}_2$  in the atmosphere, will give the molar concentration of  $\text{CO}_2$  in the solution in contact with the atmosphere. By  $P$  is understood the fraction by volume of  $\text{CO}_2$  in one atmosphere. It is assumed in such a calculation that all of the dissolved  $\text{CO}_2$  is transformed into  $\text{H}_2\text{CO}_3$ . This is probably not the fact; but since the proportion transformed cannot be ascertained experimentally, the assumption is the best that can be made. We have then as the equation for the relation between the carbon dioxide of the air and the carbonic acid of the solution,<sup>2</sup>

$$[\text{H}_2\text{CO}_3] = cP \quad (1)$$

There are also required the equations for calculating the equivalent ion concentrations of the carbonic acid.

$$\frac{[\text{H}^+] \times [\text{HCO}_3^-]}{[\text{H}_2\text{CO}_3]} = k_1 \quad (2)$$

$$\frac{[\text{H}^+] \times [\text{CO}_3^{--}]}{[\text{HCO}_3^-]} = k_2 \quad (3)$$

<sup>2</sup> Brackets around a formula signify the molar concentration of the substance thus represented.

Since  $\text{CaCO}_3$  is difficultly soluble and its saturated solution a dilute one, the equation for the solubility-product of calcium carbonate completes the required number for demonstrating the solubility of  $\text{CaCO}_3$  in the soil solution.

$$[\text{Ca}^{++}] \times [\text{CO}_3^{--}] = K \quad (4)$$

By algebraic methods, Johnston (9) derived from equations 1, 2 and 3 and the ionization equation for water,  $[\text{H}^+] \times [\text{OH}^-] = K_w$ , two other equations which express the relations of the ionic concentrations in terms of ionization constants and the molar concentration of  $\text{CO}_2$ . The evaluation of these equations was determined by him from the data produced by different workers,<sup>3</sup>

$c = 0.0441$  in pure water at  $16^\circ\text{C}$ .;  $k_1 = 3.04 \times 10^{-7}$  at  $18^\circ\text{C}$ .;  $\frac{k_1}{k_2} = 5600$ ;  $K = 0.98 \times 10^{-8}$ ; and  $K_w = 0.5 \times 10^{-14}$  at  $18^\circ\text{C}$ . The required equations are now as follows:

$$[\text{Ca}^{++}] \times [\text{CO}_3^{--}] = K = 0.98 \times 10^{-8} \quad (4)$$

$$\frac{[\text{HCO}_3^-]^2}{[\text{CO}_3^{--}]} = \frac{k_1}{k_2} \times cP = 5600 \times 0.0441 P = 247 P \quad (5)$$

$$\frac{[\text{OH}^-]}{[\text{HCO}_3^-]} = \frac{K_w}{k_1 c P} = \frac{0.5 \times 10^{-14}}{3.04 \times 10^{-7} \times 0.0441 P} = \frac{3.73 \times 10^{-7}}{P} \quad (6)$$

The dissolved  $\text{CaCO}_3$  is transformed by the  $\text{H}_2\text{CO}_3$  into  $\text{Ca}(\text{HCO}_3)_2$  and by hydrolysis of  $\text{Ca}^{++}$  there is formed some  $\text{Ca}(\text{OH})_2$ . The total ion equivalent of  $\text{Ca}^{++}$  is therefore  $[\text{Ca}^{++}] = \frac{1}{2} [\text{HCO}_3^-] + [\text{CO}_3^{--}] + \frac{1}{2} [\text{OH}^-]$  which must be satisfied by the values obtained in the three equations above. (The  $\text{HCO}_3^-$  formed by the ionization of  $\text{H}_2\text{CO}_3$  which is produced by hydrolysis as well as  $\text{Ca}(\text{OH})_2$  is not taken into account, since its numerical value is practically negligible.)

Johnston's (9) calculations from the data of Schloesing (19), have been used as the basis of the writer's determination of the solubility of  $\text{CaCO}_3$  in the soil solution. The complete solubility curve of  $\text{CaCO}_3$  developed by Johnston and Williamson (11), was plotted by them with logarithms of the pressures of  $\text{CO}_2$  as abscissae and the logarithms of  $[\text{Ca}^{++}]$  as ordinates. The sector of this curve lying between  $P = 0.0003$ , the common proportion of  $\text{CO}_2$  in country-air, and  $P = 1.0000$ , or pure  $\text{CO}_2$  at atmospheric pressure, is practically a straight line. Upon a large graph of this sector, there were interpolated along the abscissa, the values of  $\log P$  showing the variations in the soil-atmosphere; there were then read off the corresponding ordinates or values of  $\log [\text{Ca}^{++}]$ , which were used in the equations as trial values. By trial and error methods

<sup>3</sup> The data of Bohr were the basis of the calculation of  $c$ ; the value of  $k_2$  determined by Walker and Cormack is generally accepted; the value of  $k_1$  cannot be determined directly, and the ratio was calculated from the work of McCoy with  $\text{NaHCO}_3$  and  $\text{Na}_2\text{CO}_3$ . His work was conducted at  $25^\circ\text{C}$ . but Johnston uses the ratio at other temperatures as no better data are available. Seyler and Lloyd claim that 7100 is a better value for the ratio which they obtained in their investigations. The value of  $K$  was calculated from solubility data obtained by Schloesing and by Engel.

the values could then be modified until all the equations were satisfied simultaneously.

Some of Schloesing's (19) data were at pressures of  $\text{CO}_2$  within the range of the soil-atmosphere and Johnston's (9) values are therefore incorporated in table 1.

This table shows that for any pressure of  $\text{CO}_2$  in the atmosphere, there is bound to be a definite concentration of calcium in the solution, principally as  $\text{Ca}(\text{HCO}_3)_2$ , provided there is solid  $\text{CaCO}_3$  in contact with the solution.

Although the calculations have been made for pure water at a temperature of  $16^\circ$ , there is strong probability that the results may be representative of a large part of the growing season in the real soil-solution. The temperature of  $16^\circ$  is in the higher part of the range of soil-temperatures. The solubility of

TABLE 1  
Concentration of ions of  $\text{CaCO}_3$  in water at  $16^\circ\text{C}$ . in equilibrium with  $\text{CO}_2$  at various pressures

P	$[\text{Ca}^{++}]$	$1/2[\text{HCO}_3^-]$	$[\text{CO}_3^{--}]$	$[\text{OH}^-]$	pH
*0.0003	0.000579	0.000561	0.000017	$1.44 \times 10^{-8}$	8.44
*0.0005	0.000682	0.000661	0.000014	$1.00 \times 10^{-8}$	8.30
0.0010	0.000853	0.000842	0.000011	$0.63 \times 10^{-8}$	8.10
0.0020	0.001072	0.001063	0.000009	$3.96 \times 10^{-7}$	7.89
*0.0033	0.001222	0.001214	0.000008	$2.80 \times 10^{-7}$	7.74
0.0050	0.001451	0.001444	0.000007	$2.14 \times 10^{-7}$	7.63
0.0100	0.001826	0.001820	0.000006	$1.35 \times 10^{-7}$	7.43
*0.0138	0.001945	0.001940	0.000005	$1.12 \times 10^{-7}$	7.35
0.0150	0.002091	0.002086	0.000005	$1.03 \times 10^{-7}$	7.31
0.0170	0.002180	0.002175	0.000005	$0.95 \times 10^{-7}$	7.28
0.0200	0.002296	0.002291	0.000005	$0.93 \times 10^{-7}$	7.26
0.0250	0.002477	0.002472	0.000005	$0.74 \times 10^{-7}$	7.16
*0.0282	0.002541	0.002537	0.000004	$0.67 \times 10^{-7}$	7.12
0.0334	0.002726	0.002722	0.000004	$0.61 \times 10^{-7}$	7.08
*0.0500	0.003048	0.003045	0.000003	$0.47 \times 10^{-7}$	6.97
*1.0000	0.008440	0.008440		$0.31 \times 10^{-8}$	5.80

\* From Johnston's table calculated from Schloesing's data.

$\text{CO}_2$  in water increases as the temperature falls, hence should be greater during a large part of the growing season. The soluble salts in the soil-solution, according to the writer's observation, are generally below a total concentration of 0.1 N calculated in terms of  $[\text{NaCl}]$  and will reduce the solubility of  $\text{CO}_2$  slightly. Respiration of plant-organs in the soil decreases with lowering temperature and will more or less offset the increased solubility of  $\text{CO}_2$  in the solution by lessening the pressure in the soil-air at the lower temperatures.

To transform the ion concentrations  $[\text{Ca}^{++}]$  into the equivalent expression of  $\text{CaCO}_3$  in parts per million of solution, there is required the degree of ionization for each concentration. Since there were no conductivity data for calcium carbonate, Johnston (9) used the data for calcium acetate as a salt closely similar to calcium bicarbonate which forms the principal compound in the

solution. The carbonate and hydroxide are undoubtedly completely ionized. By interpolation on a large scale graph, the degrees of ionization for the different concentrations of  $[Ca^{++}]$  were obtained with reasonably close approximation. In table 2 are presented the solubility of  $CaCO_3$  in parts per million by means of the calculations.

The solubility of  $CaCO_3$  in water in contact with air containing 0.00032 part of  $CO_2$  has been measured by Wells (27) at temperatures ranging from  $0^\circ$  to  $30^\circ$ , showing a steady decrease from 81 parts per million at  $0^\circ$  to 52 parts at  $30^\circ C$ . Johnston and Williamson (11) calculated the value of  $c$  at the different determinations, and demonstrated the decrease in molar concentration of  $H_2CO_3$  with rise in temperature.

TABLE 2  
*Pressures of  $CO_2$  and corresponding solubility of  $CaCO_3$  in parts per million of solution*

P	$Ca^{++}$	IONIZATION	Ca	$CaCO_3$
			<i>mole</i>	<i>p.p.m</i>
*0.0003	0.000579	0.915	0.000631	63.1
*0.0005	0.000682	0.912	0.000746	74.6
0.0010	0.000853	0.899	0.000948	94.8
0.0020	0.001072	0.895	0.001198	119.8
*0.0033	0.001222	0.890	0.001372	137.2
0.0050	0.001451	0.885	0.001640	164.0
0.0100	0.001826	0.875	0.002086	208.6
*0.0138	0.001945	0.870	0.002231	223.1
0.0150	0.002091	0.867	0.002412	241.2
0.0170	0.002180	0.865	0.002521	252.1
0.0200	0.002296	0.862	0.002664	266.4
0.0250	0.002477	0.857	0.002890	289.0
*0.0282	0.002541	0.856	0.002965	296.5
0.0334	0.002726	0.850	0.003207	320.7
*0.0501	0.003048	0.844	0.003600	360.0
*1.0000	0.008440	0.778	0.010850	1085.0

\* From Johnston's table.

A study of the solubility of  $CaCO_3$  in lysimeters, made by MacIntyre (14), is corroborative of the theoretical principles of this discussion. Three lysimeters containing about 8 inches in depth of soil, were treated with large quantities of  $CaCO_3$  in the proportions of 8 tons, 32 tons and 100 tons per acre of surface. The drainage waters were collected for four successive years, and the total calcium determined in them and calculated as  $CaCO_3$ . The results in grams per liter of water are given in table 3.

The average concentration for the last two years is about 125 parts per million. It is clearly seen that the solubility was independent of the quantity of  $CaCO_3$  in the soil, which is of course due to the constant presence in all of them of solid  $CaCO_3$ . In the first year, some of the calcium carbonate was undoubtedly transformed into nitrate, sulfate and chloride; but by the third and fourth

years, it is reasonably sure that the solubility was essentially in the form of bicarbonate. The rate of solubility in these later years corresponds to a low percentage of  $\text{CO}_2$  in the soil-air, which would naturally be the condition in a soil with no vegetation growing in it.

The pH values in table 1 were derived from the values of  $[\text{OH}^-]$  obtained by equation 6, by using the equation for the ionization of water, which may be written  $\frac{1}{[\text{H}^+]} = \frac{[\text{OH}^-]}{K_w}$  from which we get  $\text{Log} \frac{[\text{OH}^-]}{K_w} = \text{Log} \frac{1}{[\text{H}^+]} = \text{pH}$ . The value of pH may also be obtained from equation 2 which can be transformed into  $\frac{1}{[\text{H}^+]} = \frac{\text{HCO}_3^-}{k_1 c P}$ . Therefore  $\text{Log} \frac{1}{[\text{H}^+]} = \text{Log} \frac{[\text{HCO}_3^-]}{k_1 c P} = \text{pH}$ . This latter equation shows that with a change in P, the pressure of  $\text{CO}_2$ , there must be a change in the value of pH. An increase in  $\text{CO}_2$  will lower the value of pH, that is, the alkalinity is lessened.

In the absence of other salts, the presence of  $\text{CaCO}_3$  in the soil will insure a reaction on the alkaline side of neutrality within the ordinary range of  $\text{CO}_2$  found in the soil-atmosphere, but the value of pH cannot rise above the value for the solution in contact with the free air.

TABLE 3  
*Leaching of  $\text{CaCO}_3$  from soil expressed in grams per liter of drainage water*

$\text{CaCO}_3$ IN SOIL	1914-15	1915-16	1916-17	1917-18
ions	gm.	gm.	gm.	gm.
8	0.4285	0.1615	0.1250	0.1187
32	0.3643	0.1570	0.1275	0.1232
100	0.4232	0.2175	0.1265	0.1313
Average . . . . .	0.4053	0.1887	0.1263	0.1244

#### THE EFFECT OF SOLUBLE SALTS ON THE SOLUBILITY OF $\text{CaCO}_3$ IN EQUILIBRIUM WITH $\text{CO}_2$

In common fertilizer practice there are applied to the soil phosphates of calcium; sulfates, nitrates and chlorides of potassium, sodium, calcium and magnesium; and sulfate of ammonia. The calcium salts have an ion in common with the calcium carbonate and may be expected to depress its solubility. The salts of potassium, sodium, magnesium and ammonia have no ions in common with  $\text{CaCO}_3$  and therefore tend to increase its solubility.

The extent of their influence on the soil-solution in the presence of a reserve of  $\text{CaCO}_3$  is of much interest and it is possible to indicate it approximately. The experimental data are however scarce and their use is of more value qualitatively than otherwise.

Stieglitz (23) has discussed the theory of the action of  $\text{CaSO}_4$  on  $\text{CaCO}_3$  at considerable length. His outline is followed here; but with new data.

Since  $\text{CaSO}_4$  is a difficultly soluble salt, its saturated solution will have a definite solubility-product constant in accordance with the equation

$$[\text{Ca}^{++}] \times [\text{SO}_4^{--}] = K^1$$

Harkins and Paine (8) have determined the ion concentration and solubility-product of a saturated solution of  $\text{CaSO}_4$  at  $25^\circ$ .

$$[\text{Ca}^{++}] = [\text{SO}_4^{--}] = 0.015816 \text{ and } K^1 = (0.015816)^2 = 2.503 \times 10^{-4}$$

According to Stieglitz, in a solution saturated simultaneously with  $\text{CaCO}_3$  and  $\text{CaSO}_4$ , the value of the ion concentration of calcium in the solution would be expressed by both solubility-product equations, viz.,

$$\begin{aligned} [\text{Ca}^{++}] \times [\text{CO}_3^{--}] &= K \\ [\text{Ca}^{++}] \times [\text{SO}_4^{--}] &= K^1 \end{aligned}$$

At  $25^\circ$ , Johnston and Williamson (11) have calculated the value of  $K$  to be  $0.87 \times 10^{-8}$ .

The proportion of sulfate ions to carbonate ions is expressed by the ratios

$$\frac{[\text{SO}_4^{--}]}{[\text{CO}_3^{--}]} = \frac{K^1}{K} = \frac{2.503 \times 10^{-4}}{0.87 \times 10^{-8}} = 28770$$

Therefore there is about 28,000 times as much sulfate as there is carbonate in solution simultaneously at  $25^\circ$ , and for a preliminary calculation it is assumed that the ion concentration  $[\text{Ca}^{++}]$  is wholly from the  $\text{CaSO}_4$  and is 0.015816. If the solubility-product  $K$ ,  $0.87 \times 10^{-8}$ , is divided by 0.015816, there is obtained the maximum value for the ion concentration  $[\text{CO}_3^{--}] = 0.55 \times 10^{-6}$ .

Using the equation for the concentration of bicarbonate and carbonate in equilibrium with the  $\text{CO}_2$  of the air, which is

$$\frac{[\text{HCO}_3^-]^2}{[\text{CO}_3^{--}]} = \frac{k_1}{k_2} \times cP$$

we may for convenience write it in the form

$$[\text{HCO}_3^-] = \sqrt{\frac{k_1}{k_2} \times cP \times [\text{CO}_3^{--}]}$$

The value  $\frac{k_1}{k_2}$  is still maintained at 5600, but at  $25^\circ$  the value of  $c$  is 0.0338.

The  $\text{CO}_2$  pressure  $P$  may be any pressure within the range of soil-air. Taking the highest value mentioned by Turpin (24), 0.0334, and the maximum value obtained for  $[\text{CO}_3^{--}]$  in the presence of  $\text{CaSO}_4$ , the above equation becomes

$$\begin{aligned} [\text{HCO}_3^-] &= \sqrt{5600 \times 0.0338 \times 0.0334 \times 0.55 \times 10^{-6}} \\ [\text{HCO}_3^-] &= 0.001865 \end{aligned}$$

The bicarbonate ions are twice as numerous as the calcium ions, hence  $[\text{Ca}^{++}]$  from the dissolved bicarbonate<sup>4</sup> will be 0.000932.

<sup>4</sup>It must be kept in mind that these calculations are only approximations that serve to show the direction of the reactions.



The degree of ionization of calcium acetate of equivalent concentration is 0.90 and the molar concentration of  $\text{CaCO}_3$  will be 0.00103, or 103 parts per million of solution. The presence of  $\text{CaSO}_4$  has theoretically lowered the solubility of  $\text{CaCO}_3$  under the conditions from about 300 parts per million to 100 parts per million.

The effect of the  $\text{CaSO}_4$  on the reaction of the solution can be shown by using the equation for the value of pH, viz.,

$$\frac{1}{[\text{H}^+]} = \frac{[\text{HCO}_3^-]}{k_1 \text{ cP}} = \frac{0.001865}{3.04 \times 10^{-7} \times 0.0338 \times 0.0334}$$

$$\text{Log } \frac{1}{[\text{H}^+]} = 6.63 = \text{pH}$$

The value of pH has been changed from 7.08 to 6.63 or from neutrality to acidity in this particular case.

The lowering of the value of pH can easily be seen qualitatively by shaking two solutions, one of water with solid  $\text{CaCO}_3$  alone and the other with both solid  $\text{CaCO}_3$  and solid  $\text{CaSO}_4$ . On adding an indicator, preferably cresol red, there will be noticeable a marked depression of the pink tint in the solution containing  $\text{CaSO}_4$ .

Land plaster or gypsum may have value at times in reducing the alkalinity of a soil-solution for some crops, when the presence of  $\text{CaCO}_3$  is a disadvantage, as in cases of potato scab and some root rots.

The effect of phosphates of calcium on the solubility of  $\text{CaCO}_3$  should be similar to the action of  $\text{CaSO}_4$  because there is the common ion  $\text{Ca}^{++}$  and the dicalcic as well as the tricalcic phosphate is a difficultly soluble salt in water, so there may also be a saturated solution with respect to it in the soil at times. Cameron and Seidell (4) in a study of the solubility of these phosphates used solutions saturated with  $\text{CO}_2$  at atmospheric pressure in contact with both the solid phosphate and solid  $\text{CaCO}_3$  at 25°. Their results were as follows in total Ca dissolved and total  $\text{PO}_4$ .

	CALCIUM	PHOSPHATE
40 gm. $\text{Ca}_3(\text{PO}_4)_2$ per liter.....	0.3060 gm.	Trace
40 gm. $\text{CaHPO}_4$ per liter.....	0.3130 gm.	0.0810 gm.

In the first case the molar concentration, [Ca], was 0.00765 and in the second it was 0.00782, from both phosphate and carbonate. The phosphate concentration was not estimated in the first but in the second case it was  $[\text{PO}_4] = 0.00085$ , one-half of which subtracted from 0.00782 leaves 0.00740 as [Ca] from carbonate in solution, assuming that the dicalcic phosphate is transformed into the monocalcic by the carbonic acid. For a comparison with the solubility of  $\text{CaCO}_3$  alone in water saturated with  $\text{CO}_2$  at atmospheric pressure we turn back to table 2 where the molar concentration [Ca] is 0.01085 at 16°. Since

the solubility-product constants at 16° and 25° are respectively  $0.98 \times 10^{-8}$  and  $0.87 \times 10^{-8}$ , we can estimate the molar concentration at 25° to be approximately in the ratio of 87 to 98, hence  $0.01085 \times \frac{87}{98} = 0.00963$  which is a higher concentration than the molar concentration in the presence of either of the calcium phosphates. Therefore what slight data we have show an agreement with the solubility laws for dilute solutions. Since a depression of the  $[\text{HCO}_3^-]$  causes at the same time a lowering of the numerical value of pH, we see that phosphates of calcium should lessen the alkaline effect of  $\text{CaCO}_3$  to a small extent.

Salts of potassium, sodium and magnesium which occur in fertilizer, are the chlorides, sulfates and nitrates. They have no ions in common with the  $\text{CaCO}_3$  and should in dilute solutions, increase the solubility of the solid  $\text{CaCO}_3$ , in accordance with the established principles of solubility. Numerous experiments have been recorded of solutions with high concentrations of the various salt mentioned; but scarcely any data have been found at concentrations similar to a soil-solution where the soluble salts seldom reach a concentration of 0.1 normal. Cameron and Seidell (4) studied the effects of  $\text{NaCl}$  and  $\text{Na}_2\text{SO}_4$  in which they used one or two concentrations approximating this low limit. The solutions were in equilibrium with atmospheric air, which was probably the air of the laboratory, because the solubility of the  $\text{CaCO}_3$  in the water alone was much higher than any results obtained in known cases of open air and differ with the two experiments. The solutions were in contact with solid  $\text{CaCO}_3$  at 25° for 35 days or more.

The solubility was expressed in terms of  $\text{Ca}(\text{HCO}_3)_2$ .  $\text{NaCl}$  increased the solubility from 0.1046 gm. in pure water to 0.1770 gm. per liter with the salt and  $\text{Na}_2\text{SO}_4$  increased it from 0.0925 gm. to 0.2330 gm. per liter.

No data have been found involving solutions in which potassium and magnesium salts were used in equilibrium with  $\text{CO}_2$  in air, but in pure water Cameron and his co-workers found that these salts increased the solubility of  $\text{CaCO}_3$  more than was effected by sodium salts under equal conditions.

Harkins and Paine (8) have shown that 0.02 *N* and 0.1 *N*  $\text{KNO}_3$  increased the solubility of  $\text{CaSO}_4$  from the equivalent concentration 0.0306 in water alone to the equivalent 0.0482 in the highest concentration of salt. Brönsted and Petersen (1) have also shown that dilute solutions of potassium, sodium and magnesium salts cause positive increases in the solubilities of an extensive series of difficultly soluble salts containing no ions in common. It is reasonably sure that in the soil, the solid  $\text{CaCO}_3$  which may be present, will have its solubility increased by the application of fertilizers containing salts with no ions in common with it.

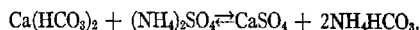
We may also consider that any increase in the solubility of  $\text{CaCO}_3$ , which is a salt of a strong base and a weak acid, necessarily produces by hydrolysis more increase in hydroxyl ions than in hydrogen ions.

Therefore the effect of fertilizer salts,  $\text{NaNO}_3$ ,  $\text{KCl}$ ,  $\text{K}_2\text{SO}_4$ , etc., will be to increase the numerical value of pH, that is, the alkalinity of the soil-solution, if  $\text{CaCO}_3$  is present.

The effect may be shown qualitatively by a simple experiment. Finely powdered  $\text{CaCO}_3$  is added to several open flasks containing respectively equal volumes of water, 0.1 *N* KCl, 0.1 *N*  $\text{NaNO}_3$  and 0.1 *N* KCl +  $\text{NaNO}_3$ . To each solution is added the same measure of an indicator, either cresol red or thymol blue. When the solutions reach equilibrium, the alkaline effects of the added salts are noticeable. It may be partly a "salt effect" on the indicator; but the low concentration of the salt and the character of the indicators used, should render such an effect nearly negligible. Furthermore, soil-extracts have shown similar comparative tints as mentioned later, whatever the cause.

Ammonium salts, of which ammonium sulfate is most commonly used in fertilizers, differ somewhat in their effects on  $\text{CaCO}_3$  in solution, from the salts of potassium and sodium. Ammonium compounds are salts of a weak base and a strong acid, or the opposite of  $\text{CaCO}_3$ , which is a salt of a strong base and weak acid.

The metathesis is represented by the reaction



The results are a neutral salt of difficult solubility and a salt readily hydrolyzed and dissociated into a weakly ionized base and acid. The first effect is to dissolve  $\text{CaCO}_3$  by forming the more soluble  $\text{CaSO}_4$ , while the formation of  $\text{H}_2\text{SO}_4$  by hydrolysis of the ammonium salt produces an acid reaction of the solution. When equilibrium is finally reached, if enough  $\text{CaSO}_4$  is formed to make a saturated solution, the result is closely like that of adding solid  $\text{CaSO}_4$  to the solution containing  $\text{CaCO}_3$ .

There are no numerical data that can be used to demonstrate this line of action, but indicators with appropriate ranges show the changes in pH values very strikingly by their varying tints.

*The action of the soluble salts on  $\text{CaCO}_3$  and on the reaction of the soil are to widen the range of pH values beyond the limits of  $\text{CaCO}_3$  alone. Salts of sodium and potassium tend to raise the pH values while calcium and ammonium salts depress them.*

This discussion of the application of solubility laws to the action of calcium carbonate in soils has been given as a preliminary to the discussion of results obtained with different fertilizers which have been applied to the plots used in the field experiments at the Massachusetts Agricultural Experiment Station.

#### THE RELATION OF SOLUBILITY LAWS TO THE FERTILIZERS USED ON THE PLOTS OF FIELD A

The plots of Field A, one of the experimental fields of the Massachusetts Agricultural Experiment Station have received a continuous fertilizer treatment since 1889. The field and its treatment have been repeatedly described in the publications of the Experiment Station, the latest one being by the writer (15).

The scheme of fertilization is shown in the diagram. Four plots lie between 1 and 6 in the field.

Diagram of Fertilizer Plots

1		6	7		8
SW	NW	W	SW	NW	W
Limed	Limed	Limed	Limed	Limed	Limed
1898	1898	1898	1898	1898	1898
1905	1905	1905	1905	1905	1905
	1919			1919	1919
Limed	Limed	Limed	Limed	Limed	Limed
1898	1898	1898	1898	1898	1898
1905	1905	1905	1905	1905	1905
1909	1909	1909	1909	1909	1909
1913	1913	1913	1913	1913	1913
	1919			1919	1919
SE	NE	E	SE	NE	E

1  
NaNO<sub>3</sub>6  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>7  
No N8  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>

Dissolved bone black and muriate of potash on all plots.

Quantities of fertilizer used annually, per acre:

Dissolved bone black	500 lbs.
Muriate of potash	250 lbs.
Nitrate of soda	300 lbs.
Sulfate of ammonia	225 lbs.

Quantities of lime applied per acre:

1898	2000 lbs.
1905	2000 lbs.
1909	5000 lbs.
1913	4000 lbs.
1919	2000 lbs.

It will be noted from the diagram that all the plots have received calcium superphosphate in the form of dissolved bone black or dissolved rock phosphate. The superphosphate is necessarily accompanied by the calcium sulfate produced in its manufacture. The other components of the soil solution vary with the different plots, and include muriate of potash, nitrate of soda and sulfate of ammonia.

All the plots have received calcium carbonate, or calcium compounds which would soon become carbonate as shown by MacIntyre (14). The quantities of lime and dates of application have been widely varied. It will be noted that parts of the plots have received no lime for 16 years, while others were heavily dressed 8 years ago, and still others sparingly dressed 2 years ago.

These variations are sufficient to test the regulatory action of CaCO<sub>3</sub> on the soil solution by a study of the solubility effects of the different fertilizers on the CaCO<sub>3</sub>.

The determination of the hydrogen-ion concentration, or the pH value, is the easiest means of studying the solubility effects, and this method has been followed for several years in comparing the soil-extracts obtained from the various fertilizer combinations.

Soil-extracts were prepared by shaking 20 gm. of air-dry soil with 200 cc. of distilled water at frequent intervals during about 1 hour. The liquid mass was then poured into a 3-inch funnel which was fitted with a small paper filter, not over 7 cm. in size. The paper and a considerable portion of the funnel were soon filled with the soil, which served as the filtering medium. The object of this practice was to reduce the adsorbing surface of the paper to as small an area as possible and prevent the selective ion adsorption which is quite noticeable when a dilute solution is filtered through a large paper filter. The filtrate was in all cases returned to the filter and passed through a second time, and if turbid, the refiltering was continued until the extract was clear. Under these conditions, the soil-extract was practically a percolate from the soil, since the small paper support must have become a negligible factor in any adsorption effect.

Some interesting differences in the behavior of the different soils were noted as the filtrations were made. All the soils are a fine sandy loam, therefore there was no difficulty in finally getting a clear and generally a colorless solution. The more acid the soils, the more freely they filtered and their extracts were like spring-water. The more alkaline the soils, the slower was the filtration and in some instances the extracts were slightly tinted.

In the measurement of pH values, colorimetric methods alone have been used. The earlier determinations were made with the indicators classified by Sørensen (22), and the standard buffer solutions recommended by Walpole (25). During the past two years, however, the solutions and indicators described by Clark (5) have been substituted with improved results. Bromocresol purple and bromthymol blue have replaced paranitrophenol and rosolic acid. Bromocresol purple gives much more brilliant gradations than paranitrophenol, and the changes of bromthymol blue are more readily matched than the tints of rosolic acid.

Precautions were steadily added as their necessity became apparent. The same glassware, especially, was used as nearly continuously as possible, in order to guard against variations in the solubility of the glass.

Gillespie (7), Sharp and Hoagland (21) and Knight (13) have very clearly shown the troubles and difficulties attending the study of pH values in soils.

The practice outlined gave consistent results each season, in the relative standing of the different soils studied; but the final combination of solutions and indicators gave slightly but positively higher pH values in all of the soils which contained  $\text{CaCO}_3$ .

The investigation during 1917 and 1918 was in the nature of an exploration. Samples were taken at irregular intervals and not simultaneously from all the plots. In 1919, other duties prevented any systematic work in the laboratory, but study of the results and of the literature caused a resumption of the work in the fall of 1920 and led to the systematic study of soil-extracts from the different plots throughout 1921.

The continuous presence of  $\text{CaCO}_3$  in the soils throughout the study was shown by the behavior of the soil-extracts when concentrated in platinum dishes. The solution from the most heavily and the recently limed soils invariably increased in alkalinity toward rosolic acid and in most cases turned phenolphthalein pink. The increased hydrolysis of  $\text{Ca}(\text{HCO}_3)_2$  by heat should produce such a result.

The results obtained in 1921 are given in table 4.

TABLE 4  
*Hydrogen-ion concentration in soil-extracts, 1921*

	MAY 3	MAY 18	JUNE 24	AUGUST 6	SEPTEMBER 2
	pH	pH	pH	pH	pH
Areas limed in 1898, 1905, 1909, 1913, and 1919					
1 NE.....	7.4	7.4	7.3	7.3	7.6
7 NE.....	7.1	7.0	7.2	7.1	6.9
8 E.....	6.6	6.5	6.6	6.8	6.7
Areas limed in 1898, 1905, 1909, 1913					
1 SE.....	7.1	7.2	7.1	6.9	7.4
6 E.....	6.3	6.2	6.0	6.2	6.4
7 SE.....	6.7	6.7	6.8	6.5	6.7
Areas limed in 1898, 1905 and 1919.....					
1 NW.....	6.8	6.9	6.8	6.7	7.0
7 NW.....	6.3	6.2	6.5	6.1	6.4
8 W.....	5.3	5.4	5.6	5.7	5.8
Areas limed in 1898 and 1905.....					
1 SW.....	6.5	6.3	6.4	6.4	6.8
6 W.....	4.5	4.5	4.6	4.5	4.8
7 SW.....	5.7	5.5	5.7	5.9	5.8

In the fertilizer scheme shown in the diagram, it will be noted that the whole field received a uniform quantity of dissolved phosphate. According to Cameron and Seidell (4), the monocalcium phosphate hydrolyzes to dicalcium phosphate and in the presence of  $\text{CaCO}_3$  there would soon be in the soil only the difficultly soluble phosphates of calcium.

The amount of dissolved phosphate employed equivalent to 80 pounds of phosphoric pentoxide,  $\text{P}_2\text{O}_5$ , per acre may be assumed to be contained in the upper layer of soil that is stirred by the harrow, when the fertilizer is first applied. The same is equally true of the other chemicals used. According to this assumption, the material is mixed with approximately 1,000,000 pounds of soil, allowing 3,000,000 pounds per acre foot. At any rate, for convenience we may take this amount as the minimum quantity of soil in which the fertilizer is held.

The amount of soil-moisture which may be present will naturally vary widely. A convenient proportion for calculation and one that is reasonably apt to be present is 10 per cent. By these assumptions we have 100,000 pounds of water per acre in which to dissolve the different fertilizers.

If all the phosphoric pentoxide were dissolved in the soil water, there would be 0.8 part in 1000 parts of water. The equivalent concentration  $[\text{PO}_4]$  would be 0.01126. Cameron and Seidell (4) secured a concentration of only 0.00085 in a solution in contact with solid  $\text{CaCO}_3$  and saturated with carbonic acid, therefore there is no doubt about the soil-solution being saturated with the difficultly soluble calcium phosphates soon after a dissolved phosphate is added to a limed soil. The slight solubility of these calcium phosphates renders their possible effects on the reaction of the soil-solution very small.

Besides 0.8 part  $\text{P}_2\text{O}_5$  in 1000 parts of soil-water, the application of the dissolved phosphate will add 1.5 parts  $\text{CaSO}_4$ . The soil-solution will not be saturated with this salt, because a saturated solution contains over 2 parts of  $\text{CaSO}_4$  in 1000 at any probable soil-temperature. Nevertheless, the effect of the dissolved phosphate will approach that of an application of  $\text{CaSO}_4$  in lowering the solubility of the  $\text{CaCO}_3$  and lessening its alkaline effect.

The other fertilizers vary with the different plots, and will be considered individually.

Potassium chloride and sodium nitrate are used simultaneously on plot 1. This plot has shown the highest value for pH consistently throughout the study, especially where lime has been applied. Both these salts are without any ions in common with each other or with  $\text{CaCO}_3$ . Their possible equivalent concentrations in 10 per cent moisture are respectively  $[\text{KCl}] = 0.033$  and  $[\text{NaNO}_3] = 0.035$ , which are the maximum concentrations practically possible if the fertilizer chemicals were pure salts. There are no data on which to base any calculations of their probable quantitative effect; but they should each increase the solubility of the  $\text{CaCO}_3$  and this is apparently true because plot 7 with KCl and without  $\text{NaNO}_3$  is consistently less alkaline than plot 1.

Ammonium sulfate was employed on plots 6 and 8 under the same conditions with respect to the dissolved phosphate and potassium chloride. The applications of lime have been varied.

If the ammonium sulfate were to react with the  $\text{CaCO}_3$  until all its  $\text{SO}_4$  had combined with Ca, the result in conjunction with the  $\text{CaSO}_4$  from the dissolved phosphate would produce a solution saturated with  $\text{CaSO}_4$ . There is no experimental evidence that such a complete interchange occurs and equilibrium probably is established with some ammonium sulfate still unchanged, although the proportion may be small. The depression of the solubility of  $\text{CaCO}_3$  is sufficient to lower the pH value below that of the other plots, which is consistently the relative position each season.

It will be noted that the pH values, while following the order of values for the solubility of  $\text{CaCO}_3$  and the different salts in pure water, are nevertheless uniformly lower in numerical quantities. This is probably due to the dilution by the extraction process. On the other hand the ten-fold dilution invariably used is not an accurate measure of the original solution, because some solubility of difficultly soluble soil-compounds will take place.

It has been invariable that concentration in platinum dishes of the soil extracts from the limed soils increases the pH values. In the extracts from plots 1 and 7, they more or less quickly become pink to phenolphthalein, while the extracts from plots 6 and 8 usually show alkalinity only to rosolic acid or bromthymol blue. This intensification of the alkalinity is partly due to expulsion of  $\text{CO}_2$  in solution and partly to the increased hydrolysis of the  $\text{CaCO}_3$  at the higher temperature. Such an intensification of the alkaline tints of indicators is strong evidence of the presence of  $\text{Ca}(\text{HCO}_3)_2$  in solution and its consequent occurrence in the soil.

On the areas which have received no lime since 1905, 1 SW, 6 W, and 7 SW, there are several indications that  $\text{CaCO}_3$  has been practically all removed. The extracts from these soils show bare traces of turbidity to ammonium oxalate, which might be attributed with good reason to the  $\text{CaSO}_4$  of the superphosphate.

In the absence of  $\text{CaCO}_3$ , the application of superphosphate may result in the formation of  $\text{AlPO}_4$  and  $\text{FePO}_4$  in addition to the tri- and di-calcium phosphates. These are all difficultly soluble salts and can affect the soil-extracts very slightly (3).

The solubility effect of ammonium sulfate is very striking under these conditions as manifested on plot 6 W.

Its action is susceptible of explanation. It is a salt of a weak base and a strong acid and consequently hydrolyzes with the formation of  $\text{NH}_4\text{OH}$  and  $\text{H}_2\text{SO}_4$ . The  $\text{H}_2\text{SO}_4$  has a much higher degree of ionization than the  $\text{NH}_4\text{OH}$ , and the solution of  $(\text{NH}_4)_2\text{SO}_4$  is therefore positively acid in its reaction. The amount hydrolyzed can be calculated by the formula for the degree of hydrolysis. The possible equivalent concentration is 0.034 *N* which is closely like that of  $\text{KCl}$  and  $\text{NaNO}_3$  in the other plots.

The equation for the degree of hydrolysis (26) of a salt with a weak base and a strong acid is for approximate calculations  $\frac{h^2 C_s}{1-h} = \frac{K_w}{K_B}$ . The degree of hydrolysis sought is *h*, the concentration of the solution is *C<sub>s</sub>*, while *K<sub>w</sub>* and *K<sub>B</sub>* are the ionization constants of water and the weak base, which in this case is ammonia. We would have on substituting numerical values (16).

$$\frac{0.034 h^2}{1-h} = \frac{0.5 \times 10^{-14}}{1.72 \times 10^{-4}} \text{ at } 18^\circ$$

which is the experimental temperature nearest the soil-temperatures.

The equation is a quadratic one and when ready for the final solution becomes for our purpose

$$h = \sqrt{\frac{0.5 \times 10^{-14}}{0.034 \times 1.72 \times 10^{-4}}}$$

The degree of hydrolysis is 0.00009 which applied to the concentration 0.034 *N* gives a concentration of  $\text{H}_2\text{SO}_4$  of 0.000003 *N*. This concentration may be



considered completely ionized, or  $\frac{1}{2} [\text{SO}_4^{--}] = [\text{H}^+] = 3 \times 10^{-6}$ . The  $\text{Log} \frac{1}{[\text{H}^+]} = 5.5 = \text{pH}$ . This is closely approximated by a solution of ammonium sulfate of this strength with methyl red as an indicator.

Numerous analyses of soil-extracts from these plots have shown that in the absence of lime there occur sulfates of aluminum, iron and manganese. The two former salts hydrolyze even more completely than ammonium sulfate. The ionization constants of these weak bases are unknown, hence their degree of hydrolysis cannot be estimated by the formula; but their pH values are 4 or less. Concentration of these soil-extracts increases their acidity intensity very noticeably toward indicators.

Like the extracts from the more recently limed areas, these appear to be diluted soil-solutions when their theoretical acidity is considered. Nevertheless, there is ample evidence that no reserve of  $\text{CaCO}_3$  is present in this area but that it has been removed owing to the solubility of  $\text{CaSO}_4$ .

The pH values found for the soil-extracts from 7 SW, appear to be due mainly to the absence of any calcium bicarbonate in the solutions, and the consequent rapid establishment of equilibrium with the  $\text{CO}_2$  in the atmosphere of the laboratory. It has been demonstrated that a solution saturated with bicarbonate at a high partial pressure of  $\text{CO}_2$ , changes very slowly toward equilibrium with a lower pressure of the gas. Two flasks containing water and solid  $\text{CaCO}_3$  were set side by side open to the atmosphere. To both were added 10 drops of cresol red indicator, which produced practically equal tints in the two solutions. Into one flask was passed  $\text{CO}_2$  until the indicator was of the deepest acid tint. Days elapsed before the indicator tints returned to equality in color. This is in marked contrast to the rapid equilibrium of a solution of carbonic acid alone, as noted by Johnston (10).

The absence of bicarbonates from the soil-extracts prepared from 7 SW, was shown when the extracts were evaporated in platinum dishes. As the concentration proceeded, the indicator either remained unchanged or turned slightly acid. If bicarbonate of calcium were present, the indicator should have turned toward alkalinity by the increasing hydrolysis of the base.

The calculated pH value of carbonic acid in water exposed to the atmosphere also compares closely with the values found in the soil-extracts. By use of equation 2 the approximate value of pH is obtained as follows:

$$\frac{[\text{H}^+] \times [\text{HCO}_3^-]}{[\text{H}_2\text{CO}_3]} = k_1 = 3.04 \times 10^{-7}$$

In a solution of carbonic acid,  $[\text{H}^+]$  and  $[\text{HCO}_3^-]$  are equal, and  $[\text{H}_2\text{CO}_3] = \text{cP}$ . Therefore the equation becomes  $[\text{H}^+]^2 = 3.04 \times 10^{-7} \times \text{cP}$ .

During the season of soil-extract examinations, the windows of the laboratory were open and as a rule the determinations were made before the open window. The value of cP is consequently assumed to be  $0.0441 \times 0.0003$  and the equation

becomes  $[H^+] = \sqrt{3.04 \times 10^{-7} \times 0.0441 \times 0.0003}$

$$\text{Log } [H^+] = -6.3022 \text{ and } \text{Log } \frac{1}{[H^+]} = 5.69 = \text{pH.}$$

This value is practically the same as those obtained in the extracts.

The value of  $H^+$  varies proportionally to the square root of the pressure  $P$  of the  $CO_2$  in the atmosphere. The acidity of the solution in the soil should be greater than that of the extracts because the  $CO_2$  must be more concentrated.

The soil-extracts from 8 W yielded about the same pH values as 7 SW, and when they were evaporated they changed indicators toward acidity. This behavior indicates that the light application of lime to this area in 1919 has been neutralized.

The soil-extracts from 1 SW were less acid than those from 7 SW and when they were evaporated, the concentrated extracts turned indicators slightly alkaline. This indicates the presence of bicarbonate of calcium in small amount, which is hydrolyzed by the heat. The probable source of this bicarbonate after 16 years with no added lime, has not yet been clearly explained by our studies.

#### SUMMARY

When solid calcium carbonate exists in the soil in contact with the soil-solution, the concentration of calcium carbonate in the solution is dependent upon the amount of carbon dioxide in the soil-air and is independent of the amount of calcium carbonate in reserve.

When chemical fertilizers are used in addition to calcium carbonate, the application of calcium phosphates, calcium sulfate and ammonium sulfate lowers the concentration of calcium carbonate in the soil-solution and lessens its alkalinity. The addition of sodium nitrate and potassium chloride increases the concentration of the calcium carbonate and the alkalinity of the solution.

The soil-extracts obtained from the limed plots of Field A have given results in accordance with these principles.

The extracts obtained from the unlimed soils showed little effect by superphosphate and potassium chloride on the pH value. The addition of ammonium sulfate produced low pH values, as though sulfates of aluminum and iron were present. Sodium nitrate when added caused the extracts to behave as though calcium bicarbonate was present.

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ACID PHOSPHATE PRODUCTION BY THE LIPMAN PROCESS:  
III. THE USE OF GREENSAND MARL AS THE INERT  
MATERIAL IN BUILDING UP SULFUR-FLOATS  
MIXTURES<sup>1</sup>

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In discussing experiment 2 (4) it was pointed out that from a physico-chemical standpoint the accumulation of phosphoric acid and sulfuric acids in the initial stage of preparing culture is the logical step in building up concentrated composts. For that purpose greenhouse soil was used as a store house with fairly good results. Since the soil has been looked upon primarily as an inert material for the accumulation of acid it was deemed possible to substitute another material for this purpose. It is important that the added materials should contain no harmful ingredients and should favor the activities of the sulfur-oxidizing organisms. Experiments conducted with greensand marl for the purpose of studying the effect of sulfur oxidation on the solubility of the insoluble potassium compounds suggested this material. These experiments, reported elsewhere (1) showed that the hydrogen-ion concentration of mixtures of greensand marl and sulfur increased very rapidly. The phosphate content of the marls should favor the reaction in the proper direction, if readily available. Besides, the potassium content of the marls would aid to the fertilizing value of the mixtures. To test out this point experiment 5 was conducted.

EXPERIMENT 5. INITIAL STAGE

A mixture of 60 gm. of marl, 25 gm. of soil and 15 gm. of sulfur was made up, the moisture-content kept as in the previous experiments and the mixture was similarly inoculated and incubated. Table 1 gives the results of the experiment.

The data presented show that the insoluble phosphates in the marls are readily set free. At this point it seems appropriate to look into the possible reactions involved in the action of sulfuric acid on the insoluble silicates. It is well known that the feldspars are relatively insoluble in acids. Still, on prolonged continuous contact with acids the silicates are attacked, liberating silicic acid. The introduction of the tertiary phosphates into a medium of

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sulfuric and silicic acid might cause the following reactions. The tertiary phosphates might be attacked setting free calcium ions; these would have to unite either with the sulfate to produce gypsum or with the silicic acid to form calcium silicate. Calcium silicate has a smaller solubility product, or ionic product as it sometimes is called, than gypsum and would be precipitated. As a result the concentration of gypsum in solution would become negligible. The concentration of the potassium sulfates and phosphates would, of course, increase, but these do not have so inhibitory an effect on the microorganisms as gypsum has. The replacement process which plays such an important rôle in mineralogical considerations, may have an effect in the greensand marl mixtures. In accordance with these theoretical considerations the greensand marl mixtures were used as the base mixture instead of the greenhouse soil.

TABLE 1  
*Conversion of insoluble phosphates of marl composted with sulfur and soil*

INCUBATION PERIOD	REACTION	SOLUBLE PHOSPHORUS PER GRAM OF MIXTURE	TOTAL P PER GRAM OF MIXTURE
<i>days</i>	<i>pH</i>	<i>mgm.</i>	<i>mgm.</i>
0	6.4	0.472	3.91
12	2.4	1.81	3.62
30	1.0	3.26	3.31

#### EXPERIMENT 6. SECOND AND THIRD STAGES

To the base mixture from experiment 5 a mixture of 100 gm. floats and 5 gm. sulfur was added. After 64 days, 91 gm. of floats alone was added, marking this point as the beginning of the third stage.

The interesting points to notice from the data are: first, the fact that in the second stage, after 5 days of incubation, the hydrogen-ion concentration reached the point where the primary phosphate makes its appearance, thus indicating a rapid conversion due to the activities of the sulfur-oxidizing organisms. At this time 33 per cent of the phosphates became soluble. This rapid rise may be explained by the accumulated acids in the initial stage, as may readily be seen from the pH of the mixture as given in table 2. Second, after 5.5 weeks free acid was accumulating again and reaction was coming fast to completion. Unfortunately, due to pressure of other work determinations were not made frequently enough to follow the reaction closely. As it was, the third stage was begun only after 9 weeks. The reaction as it may be noticed was slowing down. Still, after 44 days over 60 per cent of the total  $P_2O_5$  content (almost 12 per cent) became available. If we compare the marl series of building up of the compost with that of the soil series (4) we might at first be inclined to think that better results were secured with the soil, especially if we compare culture 1 in table 3 (4) with the marl series. There the total incubation period is 17 weeks. The total  $P_2O_5$  content was 13 per cent with 55 per cent of it available. In the marl series we have only a 12 per cent total and over 60 per

cent available, but the total incubation period is a little over 19 weeks. An analysis showed that all the sulfur had been oxidized; the culture, therefore, suffered from the lack of the driving force of the reaction. It is the author's opinion that upon repeating the series with slight modification the marl cultures might be in the lead. It should be recorded here that the marl mixtures were in a better physical condition than the soil mixtures.

TABLE 2

*Course of conversion of insoluble phosphates into soluble form in the second and third stage of the Lipman process*

INCUBATION PERIOD	REACTION	TOTAL P PER GRAM OF MIXTURE	SOLUBLE P PER GRAM OF MIXTURE	SOLUBLE PHOSPHORUS
<i>Second stage</i>				
<i>days</i>	<i>pH</i>	<i>mgm.</i>	<i>mgm.</i>	<i>per cent</i>
5	2.8	23.3	7.8	33.0
38	2.2	22.6	16.2	73.0
64	1.8	....	22.08	100.0(?)
<i>Third stage</i>				
0	3.4	54.9	17.4	32.0
44	2.8	51.38	31.29	60.9

## EXPERIMENT 7

*Course of conversion of soluble phosphates from a highly concentrated mixture to a less concentrated mixture*

Simultaneously with experiment 6 another experiment was performed with the idea of working backwards, i.e., beginning with a highly concentrated mixture and gradually diluting it with soil or marl. Mixtures were made up as indicated in table 3.

The mixtures were made up to the proper moisture-content as in the previous experiment and allowed to incubate.

Table 4 gives the results of the experiment.

The prolonged period of incubation necessary for the concentrated mixtures to reach the desired point of the reaction, namely a pH 2.8, makes this procedure an unpractical method. A step forward, however, must be recorded. In the earlier work of Lipman, McLean, Shedd, Rudolfs and those of the author of this paper, concentrated composts had to be abandoned. The only difference between their technique and that of the author was the appreciation of the moisture factor and the gain in weight of the cultures. In the cultures just described we find that number 1 had a total  $P_2O_5$  content of 18 per cent with more than 42 per cent made available and number 6 had a total  $P_2O_5$  content of 15 per cent with more than 50 per cent made available.

TABLE 3

*Composition of various sulfur-floats cultures and additions made after 9 weeks of incubation*

CULTURE NUMBER	FLOATS	SULFUR	GREENSAND MARL	GREENHOUSE SOIL	ADDITIONS MADE AFTER 9 WEEKS OF INCUBATION	
					Greenhouse soil	Greensand marl
	gm.	gm.	gm.	gm.	gm.	gm.
1	360	90	50	..	50	..
2	360	90	50	..	50	..
3	360	90	..	50	50	..
4	300	95	125	..	50	..
5	300	75	125	..	50	..
6	300	75	..	125	..	50

TABLE 4

*Course of conversion of insoluble phosphates in the first and second stage in working down high concentrated mixtures to less concentrated*

CULTURE NUMBER	AFTER 2 WEEKS' INCUBATION			AFTER 9 WEEKS' INCUBATION			AFTER 16 WEEKS' INCUBATION		AFTER 22 WEEKS' INCUBATION		AFTER 29 WEEKS' INCUBATION		
	Reaction	Total P per gm. of mixture	Soluble P per gm. of mixture	Reaction	Total P per gm. of mixture	Soluble P per gm. of mixture	Reaction	Soluble P per gm. of mixture	Reaction	Soluble P per gm. of mixture	Reaction	Total P per gm. of mixture	Soluble P per gm. of mixture
	pH	mgm.	mgm.	pH	mgm.	mgm.	pH	mgm.	pH	mgm.	pH	mgm.	mgm.
1	7.0	91.37	5.69	4.2	87.7	5.7	2.8	14.9	2.8	20.7	2.6	77.8	32.65
2	6.8	91.8	5.58	4.2	81.6	6.2	2.8	12.12	2.8	19.1	2.6	73.5	28.44
3	6.6	91.5	5.50	3.2	88.6	4.7	2.8	9.52	2.8	16.2	2.4	79.1	29.45
4	6.2	77.5	5.93	3.2	74.6	7.4	2.8	15.72	2.8	22.3	2.4	64.4	28.15
5	6.4	76.6	5.96	3.6	72.6	6.4	2.8	12.6	2.8	20.4	2.6	65.7	28.44
6	6.3	77.2	5.87	3.8	73.75	6.4	2.6	18.04	2.6	23.9	2.4	64.0	32.89

## CONCLUSIONS AND SUGGESTIONS

The Lipman process of making acid phosphate by biological oxidation of sulfur seems to have practical possibilities. The best method of obtaining acid phosphate of a high total and also a high percentage of soluble phosphates is to start out with a dilute mixture and gradually build it up.

The suggestions which are to be made and, which the author hopes to try out if the opportunity will avail itself, are as follows:

1. Greensand marl should be used as the inert material for the initial stage with a small addition of soil rich in organic matter.
2. The sulfur content should be increased in the initial stage, thus making possible a larger accumulation of acid.
3. Simultaneously with the base mixture the second stage should be incubated and the sulfur content of this mixture should also be increased. This will appreciably cut down the time period in the second stage.
4. Only at the third stage should floats alone be added in an amount necessary to take care of the excess of sulfur added in the initial and second stages.

5. The moisture-content should be carefully kept up as given in the section on the effect of moisture content, as given in the first paper of this series (3).

6. Proper aeration should be provided for. If possible, mechanical aeration as outlined in the experiment on aeration (2) or something similar, should be practised.

7. The use of catalytic and stimulating agents found by McLean to be of value should be tried out, including some which were found by the author to be stimulating to *Thiobacillus thiooxidans* in culture solution, as reported (1).

8. A study should be made on the tolerance limits by the sulfur-oxidizing organisms to the salts found in a water extract of the mixtures.

It is the conviction of the author that, with proper handling, acid phosphate production by biological means may be accomplished on a practical basis. There is no doubt that a mixture with a total  $P_2O_5$  content of 16 to 18 per cent of which 60 to 75 per cent or even more is available, could be made in three months.

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# SOIL ACIDITY AS MEASURED BY SUGAR INVERSION, THE TRUOG TEST AND THE HYDROGEN-ION CONCENTRATION AND ITS RELATION TO THE HYDROLYSIS OF ETHYL ACETATE<sup>1</sup>

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It is generally recognized that acid soils contain soluble acids, as indicated by the hydrogen-ion concentration of the soil solution, and insoluble acids in the form of acid silicates and organic acids. There is need for a single measure of both of these forms of acids. The hydrogen-ion concentration is generally supposed to indicate the strength or degree of dissociation of the soluble acids and not the acidity of the insoluble or undissociated acids. However, it is probable that the hydrogen-ion concentration of most acid soils depends to a considerable extent on the amount and strength of the difficultly soluble acids in the soil. With this assumption, there should be a correlation between the hydrogen-ion concentration of the soil solution, the amount of sugar inverted by the soil and the degree of acidity indicated by the Truog test.

The sugar inversion method was studied because the rate of sugar inversion is generally considered to be a measure of the hydrogen-ion concentration. However, Rice and Osugi (7) and Osugi (4) reported that sugar inversion is influenced by the insoluble acids as well as by the hydrogen-ions in the soil solution. Other investigators (1) have advanced evidence to show that the catalytic effect of acids on the inversion of cane sugar is due partly to the hydrogen-ions and partly to the undissociated acid. The data of Rice and Osugi (7) show that the inversion of sugar is much greater in soil suspensions than in water extracts of the same soil. From later studies (4) Osugi concluded that the insoluble acid silicates in the soil suspension produce the greater part of the sugar-inverting power of acid soils. From this it appears that the sugar inversion method should give a good indication of soil acidity. Assuming that the hydrogen-ion concentration of the soil solution is influenced by the amount and strength of the insoluble acids, the results of the sugar inversion method should correlate fairly well with the hydrogen-ion concentration of the soil solution.

<sup>1</sup> Published with the permission of the Director of the Wisconsin Agriculture Experiment Station.

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The Truog test (9) was included in this investigation because it is widely used as a practical test. Truog (8) has presented data showing that the degree of acidity indicated by this test is a resultant of the acidity and amount of the "active" soil acids. "Active" acids include all of the soluble and a considerable part of the insoluble acids. The results of this test should bear a fairly close relationship to the results of the sugar inversion method for the results of both methods are influenced by the soluble and insoluble acids. The Truog test should correlate fairly well with the hydrogen-ion concentration of the soil but the correlation between these two tests cannot be expected to be as close as the correlation between the results of the Truog test and the sugar inversion method.

Johnson (3) studied the relation between the hydrogen-ion concentration of fifty soils, the Veitch lime requirement and the degree of acidity indicated by the Truog test. He found very little relation between the Veitch lime requirement and the hydrogen-ion concentration. He states that the Truog method gave results which were a combination of the Veitch lime requirement and hydrogen-ion concentration.

#### EXPERIMENTAL

The acidity of forty-two soils was determined by the three methods mentioned above. The hydrogen-ion concentration was determined colorimetrically and electrometrically. At first some difficulty was experienced in getting the two methods to check satisfactorily. Later the difficulty was overcome. Only the results of the electrometric method are recorded in this paper. Readings of the electromotive force were made with an improved Leeds and Northrup potentiometer. Duplicate determinations agreed very closely and the results are accurate to pH 0.05. The hydrogen-ion concentration of the displaced soil solution (5) was found to be practically the same as that of a 1:2 water extract. This result does not agree with results secured by Plummer (6). He reported a higher hydrogen-ion concentration in the soil solution of an acid soil than in the water extract of the same soil.

The sugar inversion method was essentially the same as that used by Rice and Osugi (7). Fifty cubic centimeters of a 5 per-cent cane sugar solution were added to a flask containing 10 gm. of 20 mesh air-dry soil. The flasks were then placed in a water bath and a temperature of 80°C. maintained for three hours. During this time the flasks were frequently shaken to keep the soil in suspension. Upon removal from the water bath, distilled water was added to replace the small amount lost by evaporation. The suspension was then filtered and the invert sugar determined by titration. The results recorded are from single determinations. Duplicate determinations were made on several soils and the results agreed very closely.

The Truog test (9) was made in the usual manner.

The results secured with the three methods on twenty-three soils are given in table 1 and are shown graphically in figure 1. The soils are arranged in order of their acidity as indicated by the Truog test. It is at once evident that there is a good correlation between the results secured by the three methods. The correlation is best between the amount of sugar inverted and the acidity indicated by the Truog test. This is to be expected since the results of both methods are influenced by the quantity and strength of both the soluble and insoluble acids present. The hydrogen-ion concentration shows a very good relationship to the results of the other two methods. If

TABLE 1

*Soil acidity as indicated by sugar inversion, the Truog test and the hydrogen-ion concentration*

SOIL NUMBER	SUGAR INVERTED	HYDROGEN-ION CONCENTRATION	REACTION BY TRUOG TEST
	gm.	pH	
1	Trace	6.83	Very slight -
2	0.021	6.01	Very slight
3	0.017	5.72	Very slight +
4	0.017	6.67	Slight -
5	0.025	6.00	Slight
6	0.023	5.75	Slight
7	0.017	6.03	Slight +
8	0.033	6.15	Slight +
9	0.022	5.45	Slight ++
10	0.032	5.70	Medium --
11	0.030	5.77	Medium -
12	0.045	5.23	Medium
13	0.043	5.14	Medium +
14	0.064	5.50	Medium ++
15	0.053	5.34	Medium ++
16	0.080	5.14	Strong --
17	0.064	5.30	Strong -
18	0.110	5.16	Strong
19	0.127	5.22	Strong +
20	0.120	5.06	Strong +
21	0.133	4.73	Very strong -
22	0.186	4.85	Very strong +
23	0.350	4.32	Very strong +

soils of the same type and texture but with different field treatments are compared the results should correlate much better than when a large number of soils are selected without regard to type or texture.

In order to determine whether or not the correlation is better between soils of exactly the same type but having different field treatments, a second series of determinations was made. Nineteen soil samples, representing eight soils with two or more different field treatments were secured and the acidity determined by the three methods. The results are given in table 2 and graphically in figure 2.

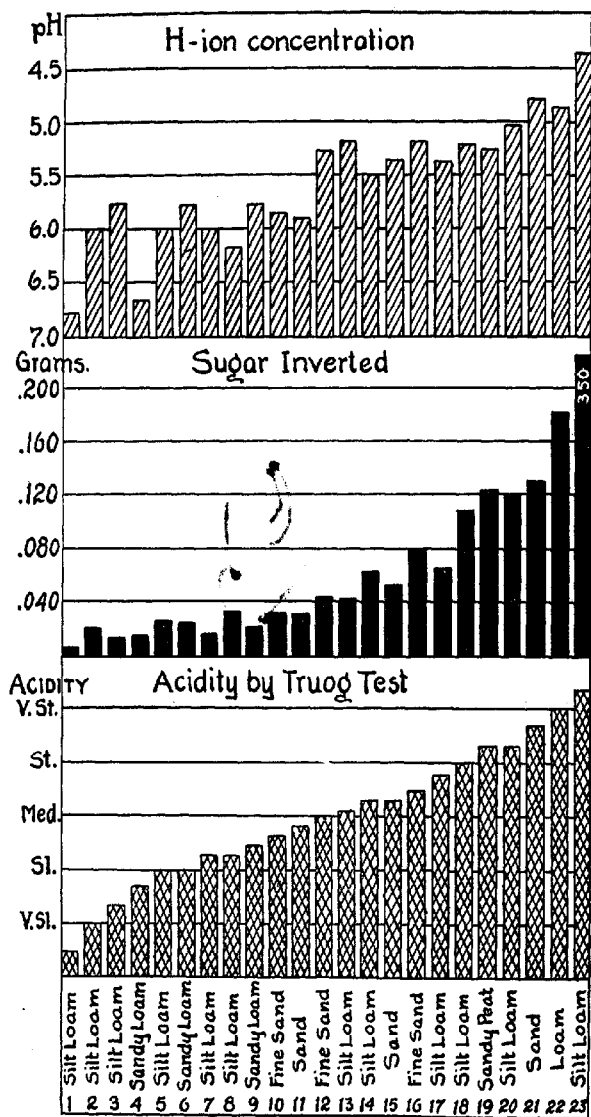


FIG. 1. COMPARISON OF RESULTS OBTAINED ON 23 DIFFERENT SOILS WITH SOIL ACIDITY METHODS INDICATED

In every instance, with one exception, the three methods indicate the same differences in the acidity of the soils due to the different treatments. It is difficult to make a quantitative comparison but a careful analysis of the results indicates, as is also evident in figure 1, that the correlation is best between the results of the sugar inversion method and the Truog test.

TABLE 2

*Soil acidity as indicated by sugar inversion, the Truog test and the hydrogen-ion concentration*

SOIL	TREATMENT	SUGAR INVERTED	REACTION BY TRUOG TEST	HYDROGEN- ION CON- CENTRATION
		gm.		pH
Sand.....	Virgin	0.017	Slight +	5.79
	Cropped	0.026	Medium	5.50
Sandy loam.....	Virgin	Trace	Very slight -	7.53
	Cropped	0.000	Not acid	8.09
Sandy loam.....	Virgin	0.040	Medium +	5.60
	Cropped	0.010	Slight	6.42
Silt loam.....	Virgin	0.024	Medium	5.62
	Cropped	0.021	Slight +	5.88
Silt loam.....	Virgin	0.018	Slight	5.61
	Cropped	0.008	Very slight	6.48
Sand.....	No lime	0.022	Slight	5.50
	Limed	0.000	Very slight -	6.90
Sand.....	Virgin	0.018	Slight +	6.42
	Cropped	0.031	Medium +	5.92
	Limed	Trace	Very slight	6.67
Silt loam.....	Virgin	0.092	Very strong	5.22
	Cropped	0.064	Strong	5.18
	Limed	0.024	Medium	6.00
	Lime and manure	0.014	Slight +	6.06

## DISCUSSION

All of the results reported in tables 1 and 2 indicate that there is a very close relation between the degree of acidity indicated by the Truog test and the amount of sugar inverted. When the acidity is low the Truog test is probably nearly as sensitive to small differences in acidity as is the sugar inversion method. When the acidity is high it is difficult to recognize small differences in the intense color produced with the Truog test, and in such cases the sugar inversion method is better for showing differences. Doubtless the Truog test could be readily modified, by using less chemicals, to show greater differences with strongly acid soils.

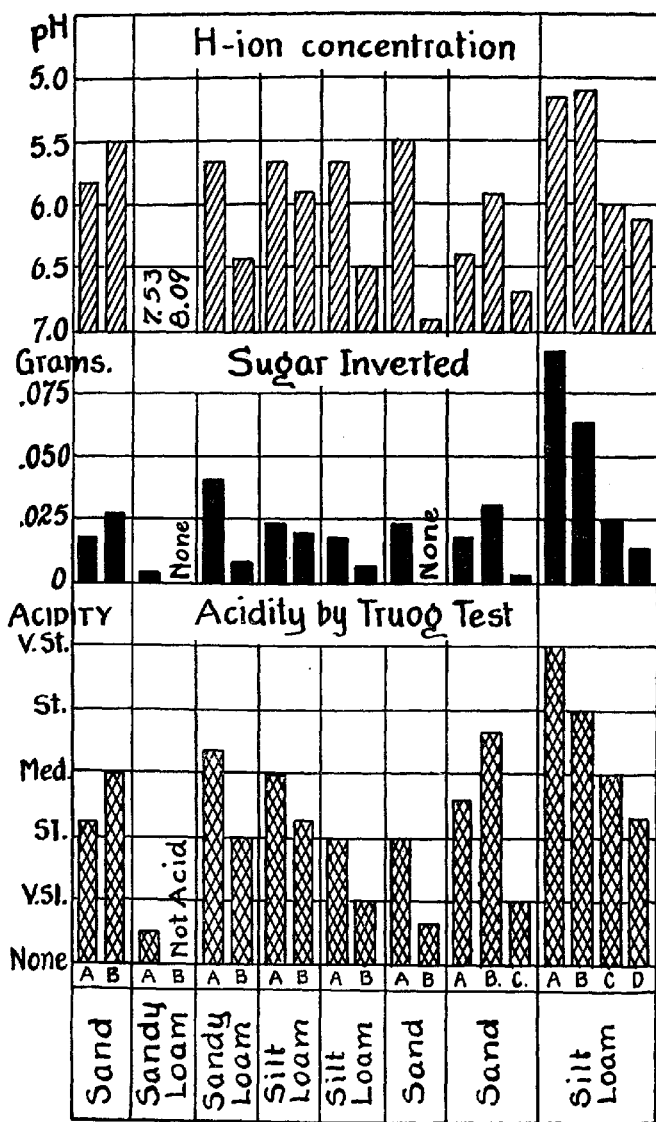


FIG. 2. COMPARISON OF RESULTS OBTAINED ON 8 SOILS VARIOUSLY TREATED WITH SOIL ACIDITY METHODS INDICATED

In general the correlation between the degree of acidity indicated by the Truog test and the hydrogen-ion concentration is good. The reason it is not better given by Truog (8) and Johnson (3) is that the Truog test is a measure of the amount of acids in the soil as well as their avidity.

There is a good general correlation between the amount of sugar inverted and the hydrogen-ion concentration of the soil. This relationship is just as close as that existing between the hydrogen-ion concentration and the results of the Truog test. These results do not agree with those reported by Rice and Osugi (7) for they failed to find any correlation between sugar inversion and the hydrogen-ion concentration. The correlation shown in figures 1 and 2 would doubtless have been better if the hydrogen-ion was the only catalyzer of sugar inversion.

The work of Rice and Osugi and similar experiments performed in this laboratory prove conclusively that the greater part of the inverting action of soil suspensions is due to the presence of the solid phase. For instance, they found that a soil suspension inverted 0.098 gm. of cane sugar while an extract of the same soil inverted only 0.002 gm. Osugi (4) states that the hydrogen-ion concentration of the soil solution is not sufficient to account for the inverting action of soils without assuming a higher concentration of hydrogen-ions about the soil particles than in the mass of the soil solution. He considers acid aluminum silicates to be the cause of the inversion.

In order to determine whether the inverting power of acid soils is due to acid silicates or to a higher concentration of hydrogen-ions around the particles than in the mass of the soil solution, additional experiments were made.

The hydrolysis of ethyl acetate as well as the inversion of cane sugar is catalyzed by hydrogen-ions. So far as is known to the authors, hydrogen-ions are the only catalysts of the hydrolysis of ethyl acetate. Conner (2) has used the hydrolysis of ethyl acetate as a means of determining soil acidity. If in a study of this method the results were found to be similar to those secured with the sugar inversion method, one could only conclude that the same catalyst was influencing both reactions. No information would be obtained as to whether this catalyst consisted of acid silicates or a high concentration of hydrogen-ions around the soil particles. However, if the results secured by the two methods differed considerably, one could conclude that different catalysts influenced the reactions or that some catalyst was influencing one reaction and not the other.

Rice and Osugi (7) have offered the following experimental results to prove that the inversion is largely due to the presence of the soil particles. (a) A suspension of acid soil inverts several times as much sugar as an extract of the same soil. (b) Inversion is greater when a soil is kept in suspension by continual shaking than when it is permitted to settle out in the flask. These results have been confirmed by the authors. Similar experiments were then made studying the hydrolysis of ethyl acetate. Soils 5, 12, 17, 20 and 22 of table 1 were used in these experiments. Using a 5-per-cent ethyl acetate



solution the amount of hydrolysis was determined in a water extract, in a soil suspension with continual shaking and in a soil suspension without shaking. The ratio of soil to water in the suspensions was 1:10. For the extract a ratio of 1:2 was used. After hydrolysis had been allowed to go on for 50 hours at room temperature, 50 cc. of the filtered extract was titrated with 0.05 *N* NaOH. The results are given in table 3.

These results are the reverse of those secured with the sugar inversion method. Hydrolysis was apparently greatest in the water extract and least in the soil suspension that was continually shaken. The low results secured with the soil suspensions may have been due partly to the neutralization of the acetic acid, by acting on minerals of the soil. It is, however, not probable that this would account for all the difference in the order of results

TABLE 3

*The hydrogen-ion concentration, sugar inverted and NaOH required to neutralize acidity, developed by the hydrolysis of ethyl acetate in soil suspensions*

SOIL	HYDROGEN- ION CON- CENTRATION	SUGAR INVERTED	0.05 <i>N</i> NaOH TO NEUTRALIZE ACID IN 100 CC.		
			1:2 Extract	Suspension (shaken)	Suspension (not shaken)
	<i>pH</i>	<i>gm.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>
Silt loam .....	6.00	0.025	3.40	0.90	1.80
Fine sand .....	5.23	0.045	5.50	4.50	5.60
Silt loam .....	5.30	0.064	5.40	3.30	3.60
Silt loam .....	5.06	0.120	6.30	3.80	4.40
Loam .....	4.85	0.186	5.10	4.50	5.80

secured by this method and the sugar inversion method. From the results secured it is evident that the high inverting power of a soil suspension as compared to a water extract is not due to a higher concentration of the hydrogen-ions around the soil particles than in the soil solution. If that were the case the two methods would have given similar results since both reactions are catalyzed by hydrogen-ions. The results are readily explained as follows: Both reactions are catalyzed by hydrogen-ions but sugar inversion is also catalyzed by acid silicates which cause most of the inversion in soil suspensions.

Additional studies are being made of the ethyl acetate method but the early results here reported indicate that the concentration of hydrogen-ions around the soil particles is not greater than in the mass of the soil solution. The results also indicate that the hydrolysis of ethyl acetate is not catalyzed by acid silicates as is the inversion of cane sugar.

## SUMMARY

1. The results secured by the hydrogen-ion determination, the sugar inversion method, and the Truog soil acidity test were compared on forty-two soils.
2. There was a fairly good correlation between the results of the three methods. The Truog test and the sugar inversion method correlate very well with each other and in a general way both correlate fairly well with the hydrogen-ion concentration.
3. Acid silicates catalyze the inversion of cane sugar and cause the greater part of the sugar inversion in suspensions of acid soils.
4. Acid silicates do not catalyze the hydrolysis of ethyl acetate in soil suspensions.

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## STUDIES ON THE TOXIC PROPERTIES OF SOILS<sup>1</sup>

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Since Wheeler and his associates carried out their extensive investigations at the Rhode Island Station on so-called acid soils, students of soil fertility problems have become more or less familiar with the bad effects produced on the soil when sulfate of ammonia is used continuously for a period of years without the addition of lime. It has become customary to say that this practice results in an acid soil, which is more or less toxic to most farm crops.

The long continued use of ammonium sulfate on Hagerstown loam at Pennsylvania State College and on Sassafra loam at this station has given rise to soils quite different in chemical and physical properties from the original soils.

During the past 25 years much has been written about acid soils and many causes of this unfavorable condition have been suggested, but with all that has been said and done there is not yet unanimity of opinion as to the cause of acidity, nor as to the identity of the substance or substances which produce the toxic effects.

Mineral and organic acids, acid salts, soluble aluminum compounds, the removal of basic materials by crops and by drainage waters have been suggested as causes of unproductive acid soils.

Recently considerable attention has been given to soluble aluminum salts as a possible cause of much of the trouble heretofore attributed to "acidity." Those who have followed this phase of the subject at all are already familiar with the work of Abbott (1), Connor (2), Hartwell (4), Denison (3), Mirasol (6), Miyake (7), Ruprecht (9), and others.

Without going into a complete bibliography of the subject, these references will serve to indicate something of the work that has already been done.

In our field work at this station on the availability of different nitrogenous materials, ammonium sulfate is used without lime on Plot 11A and with lime on Plot 11B. The results of the first 10 years have been published and may be referred to for the details of the experiment (5).

Now after 15 years of this treatment the soil on 11A has become so "acid" or toxic as to entirely inhibit the growth of ordinary farm crops. It does how-

<sup>1</sup> Paper No. 79 of the Journal Series, New Jersey Agricultural Experiment Stations, Department of Soil Chemistry and Bacteriology.

This paper will appear in *RUTGERS COLLEGE STUDIES*, vol. 1.

ever support a fair covering of sour grass (*rumex acetosella*) during the early summer and later a good covering of crab grass (*digitaria sanguinalis*). Plate 1 illustrates these conditions. The difference in treatment has likewise resulted in distinct physical differences in the two soils. The soil from 11A has a greater water-holding capacity, is closer and finer textured and when made into briquettes has a greater breaking strength than soil from 11B.

Suspecting that the trouble in this case might be due in part to soluble salts of aluminum, it was decided to make a study of the soil from both the limed and the unlimed plots, with a view to clearing up this point. Representative samples were collected from the two plots in the fall of 1921 and after being prepared in the usual way the determinations shown in table 1 were made.

TABLE 1  
*Analysis of soil from limed and unlimed plots receiving ammonium sulfate*

	PLOT 11A	PLOT 11B
Lime requirement (Veitch method).....	2,400 pounds CaO per acre	400 pounds CaO per acre
pH values.....	4.7	6.4
	per cent	per cent
Water-holding capacity.....	38.700	30.1
Total nitrogen.....	0.093	0.074
Total P <sub>2</sub> O <sub>5</sub> .....	0.111	0.112
Total carbon.....	1.230	1.110
Total Al <sub>2</sub> O <sub>3</sub> , Fe <sub>2</sub> O <sub>3</sub> and P <sub>2</sub> O <sub>5</sub> by H <sub>2</sub> SO <sub>4</sub> .....	6.440	4.140
Fe <sub>2</sub> O <sub>3</sub> .....	1.860	1.210
Al <sub>2</sub> O <sub>3</sub> (by difference).....	4.460	2.820
Total SO <sub>3</sub> .....	0.058	0.017

These figures reveal certain differences which may have a more or less important bearing on the problem. There is a pronounced difference in soil reaction, the soil from 11A showing a pH value of 4.7 and that from 11B a pH value of 6.4. By the Veitch method 11A shows a lime requirement of approximately 2½ tons per acre, while 11B is near the neutral point. It is significant that the water-holding capacity, total nitrogen and total carbon are all higher for 11A than for 11B. This would seem to indicate a more rapid disappearance of organic matter from the limed than from the unlimed plot. There is likewise a higher percentage of iron and aluminum in soil from 11A than in that from 11B. There may be some significance in the higher percentage of sulfur in 11A than 11B. Further reference will be made to this.

#### WATER-SOLUBLE IRON AND ALUMINUM COMPOUNDS

Following out the suggestion that soluble aluminum compounds might be partly responsible for the toxic condition of the soil on plot 11A, 5 kgm. of the dry soil were placed in a lysimeter made by inverting a bell glass having

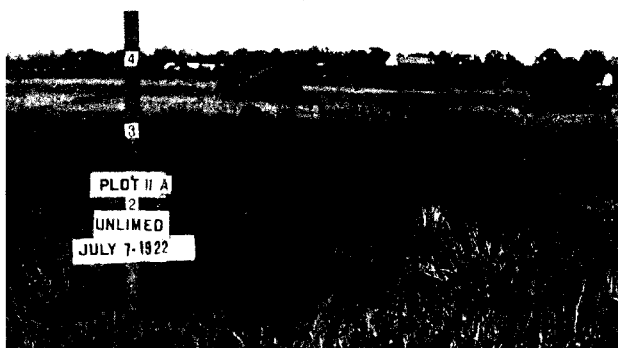


FIG. 1. UNLIMED SULFATE OF AMMONIA PLOT  
Sour grass and crab grass come in where timothy should grow



FIG. 2. LIMED SULFATE OF AMMONIA PLOT  
A good crop of timothy

PLATE 2

FIG. 1. CULTURE-SOLUTION, SERIES 1

1. Extract from 11A.
2. Extract from 11A + 0.2 gm.  $\text{NaNO}_3$ .
3. Extract from 11B.
4. Extract from 11B + 0.2 gm.  $\text{NaNO}_3$ .
5. Extract from 11A + lime water.
6. Extract from 11A + 0.5 gm.  $\text{KH}_2\text{PO}_4$ .
7. Extract from 11B + 0.5 gm.  $\text{KH}_2\text{PO}_4$ .
8. Control solution.

FIG. 2. CULTURE-SOLUTION, SERIES 2

1. Control solution.
2. Extract from 11A.
3. Extract from 11B.
4. Extract from 11A + lime water, pH 6.9.
5. Extract from 11A + lime water, pH 8.4.
6. Extract from 11A +  $\text{NH}_4\text{OH}$ .
9. Extract from sulfur plot 5, limed.
10. Extract from sulfur plot 10, unlimed.
11. Extract from sulfur plot 10 + lime water.
12. Extract from sulfur plot 10 + acid phosphate.

FIG. 3. CULTURE-SOLUTION, SERIES 3

1. Control solution.
2. Extract from plot N, unlimed.
3. Extract from plot N +  $\text{CaH}_4(\text{PO}_4)_2$ .
4. Extract from plot N + lime water.
5. Extract from plot N +  $\text{NH}_4\text{OH}$  treatment.
6. Extract from plot N +  $\text{NH}_4\text{H}_2\text{PO}_4$ .
7. Extract from plot N +  $\text{KH}_2\text{PO}_4$ .
8. Extract from plot M, limed.

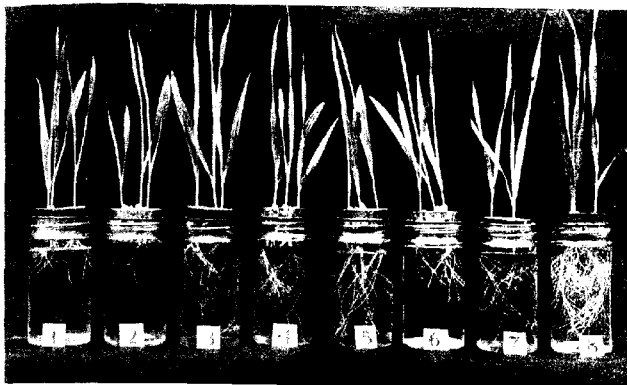


FIG. 1

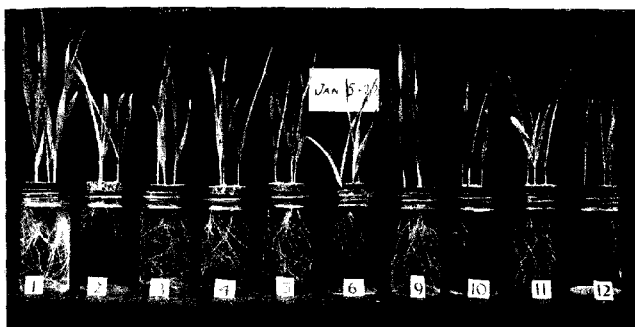


FIG. 2

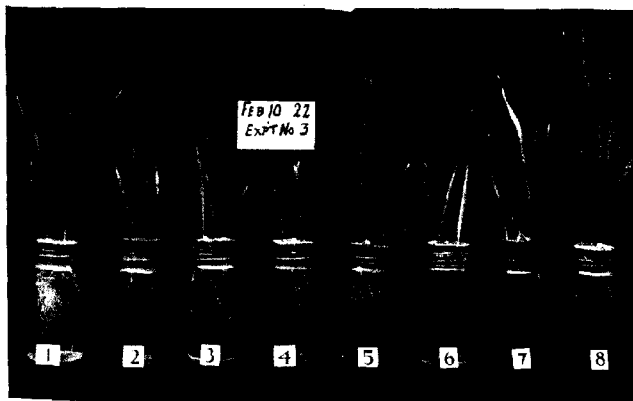


FIG. 3



### PLATE 3

FIG. 1. CULTURE-SOLUTION, SERIES 3, CONTINUED

1. Control solution.
9. Extract from plot 11A, unlimed.
10. Extract from plot 11A +  $\text{CaH}_4(\text{PO}_3)_2$ .
11. Extract from plot 11A +  $\text{NH}_4\text{OH}$  treatment.
12. Extract from plot 11A +  $\text{NH}_4\text{H}_2\text{PO}_4$ .
13. Extract from plot 11A +  $\text{KH}_2\text{PO}_4$ .
14. Extract from plot 11A 2nd liter leachings.
15. Extract from plot 11A 3rd liter leachings.
16. Extract from plot 11A + 50 cc. control solution.
20. Extract from plot 11B, limed.

FIG. 2. CULTURE-SOLUTION, SERIES 4

1. Control solution.
2. Extract from plot 11A, the solution used for culture 9, plate 3 fig. 1, + ammonia treatment.
3. Extract from plot 11A, the solution used for culture 10, plate 3 fig. 1 + ammonia treatment.
4. Extract from plot 11A and ammonia treatment + Fe.
5. Extract from plot 11A and ammonia treatment + Fe and  $\text{P}_2\text{O}_5$ .
6. Extract from plot 11A with ammonia treatment.
7. Extract from plot 11A evaporated to dryness and ignited.
8. Extract from plot 11B treated as in No. 7.
9. Extract from plot 11A unlimed.

FIG. 3. CULTURE-SOLUTION, SERIES 4, CONTINUED

1. Control solution.
9. Extract from plot 11A, unlimed.
10. Like No. 5 (Fig. 2) + 0.01 gm. aluminum sulfate.
11. Like No. 5 (Fig. 2) + 0.025 gm. aluminum sulfate.
12. Like No. 5 (Fig. 2) + 0.05 gm. aluminum sulfate.
13. Like No. 5 (Fig. 2) + 0.10 gm. aluminum sulfate.
14. Like No. 5 (Fig. 2) + 0.20 gm. aluminum sulfate.
20. Extract from plot 11B, limed.
21. Like No. 5 (Fig. 2) +  $\text{H}_2\text{SO}_4$  to pH 4.6.
22. Like No. 5 (Fig. 2) +  $\text{H}_2\text{SO}_4$  to pH 4.2.
23. Like No. 5 (Fig. 2) +  $\text{H}_2\text{SO}_4$  to pH 3.5.

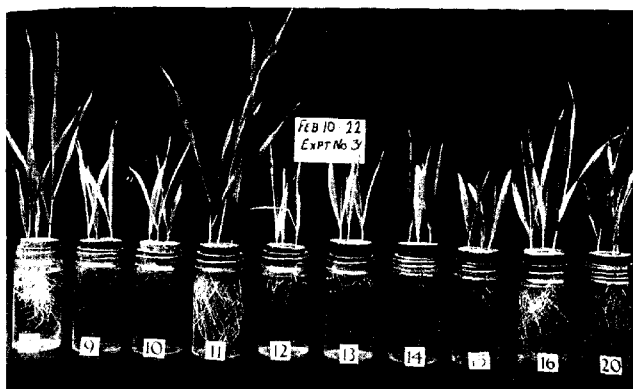


FIG. 1

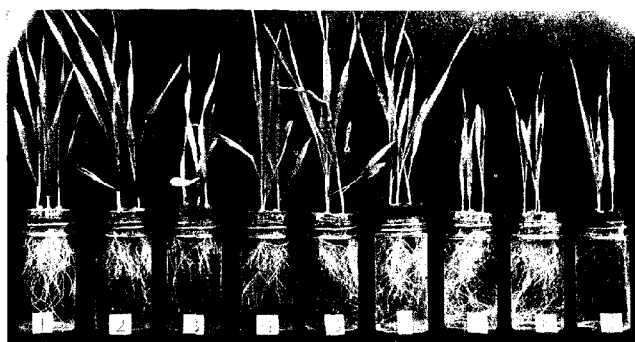


FIG. 2

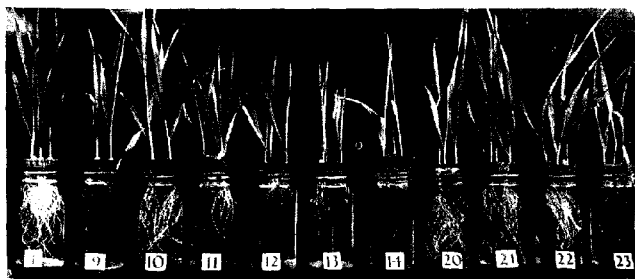


FIG. 3

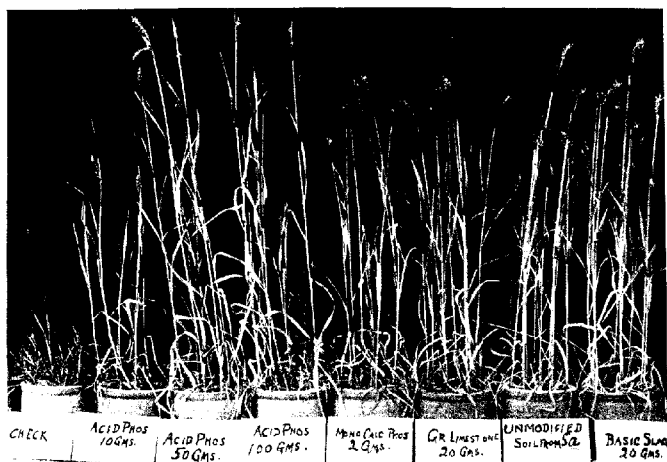


FIG. 1. POT EXPERIMENT, SERIES 2



FIG. 2. POT EXPERIMENT, SERIES 3

an opening at the top, and percolating distilled water through the mass of soil until the leachings amounted to one liter. The extract thus obtained was used for determining the soluble aluminum and other constituents, and also as a culture medium for growing plants. A similar extract was obtained from 11B for comparison. The analytical results for the two plots are reported in table 2.

From these figures it will be observed that the first liter of extract from 11A yielded 32.2 mgm. of combined iron and aluminum oxides, the greater part of which is alumina, whereas the liter of extract from 11B yielded only a trace of the combined oxides. The second liter from 11A yielded 9 mgm. and the third 7.5 mgm. Thus the three liters of extract yielded soluble iron and aluminum compounds equivalent to nearly 50 mgm. of the combined oxides,

TABLE 2  
*Analysis of a 1-liter water-extract from 5 kgm. dry soil*

MINERALS	PLOT 11A	PLOT 11B
	mgm.	mgm.
$\text{Fe}_2\text{O}_3 \cdot \text{Al}_2\text{O}_3$ .....	32.2	Trace
$\text{Fe}_2\text{O}_3$ .....	4.0	.....
$\text{Al}_2\text{O}_3$ .....	28.2	.....
$\text{CaO}$ .....	95.5	70.80
$\text{P}_2\text{O}_5$ .....	1.97	4.77
$\text{Fe}_2\text{O}_3 \cdot \text{Al}_2\text{O}_3$ (2nd liter of leachings) .....	9.00	.....
(3rd liter of leachings) .....	7.50	.....

nearly all of which was alumina. In this connection it may be pointed out that 11A furnished less than half as much water-soluble phosphoric acid as 11B. This may be due to the presence of the large amount of soluble aluminum compounds in the soil from 11A, which would tend to render the phosphoric acid insoluble.

Although the lime requirement for 11B is much less than for 11A, the liter of extract from the latter yielded 95.5 mgm. of lime ( $\text{CaO}$ ), whereas an equal amount of extract from 11B yielded only 70.8 mgm. of  $\text{CaO}$ . This seeming contradiction may be the result of the action of the sulfuric acid radical on the calcium of the soil silicates with the formation of calcium sulfate, which on account of the physical condition of the soil may not be leached out as rapidly as it is from 11B. The higher percentage of  $\text{SO}_3$  found in 11A would seem to lend support to this view.

However this may be, it is certain that the lime which has been applied to 11B has rendered the aluminum less soluble on this plot than it is on 11A.

## CULTURE-SOLUTION WORK

*Culture solution series 1*

As already noted, culture-solution work was carried out with the extract prepared by treating 5 kgm. of the soil with sufficient distilled water to obtain 1 liter. This extract was used without modification, as a culture medium for growing barley plants and also with certain modifications as hereafter indicated. An extract made in the same way from the soil of 11B was used for comparison. Table 3 shows the treatments together with the pH values of the solutions at the beginning and at the end of the experiment, and the relative weights of the crops, when dried.

TABLE 3  
*Series 1; Modified soil extract as a culture solution—reaction of solution and relative weights of crop*

CULTURE NUMBER		REACTION		RELATIVE WEIGHTS	
		Initial	Final	Tops	Roots
		pH	pH		
	Extracts from:				
1	Plot 11A.....	4.7	4.7	82.8	68.2
2	Plot 11A + 0.2 gm. NaNO <sub>3</sub> .....	4.7	4.8	64.2	56.8
3	Plot 11B.....	6.8	6.4	77.7	114.1
4	Plot 11B + 0.2 gm. NaNO <sub>3</sub> .....	6.9	7.1	64.9	75.0
5	Plot 11A + 13.5 cc. lime water.....	6.8	6.8	71.1	83.8
6	Plot 11A + 0.5 gm. KH <sub>2</sub> PO <sub>4</sub> .....	3.8	4.2	65.7	70.0
7	Plot 11B + 0.5 gm. KH <sub>2</sub> PO <sub>4</sub> .....	5.4	5.5	63.8	106.9
8	Shive's 3 salt nutrient solution (R <sub>3</sub> C <sub>2</sub> ).....	4.6	4.7	100.0	100.0

The weights of the plants grown in a 3-salt nutrient solution (Shive's R<sub>3</sub>C<sub>2</sub>)<sup>2</sup> were taken as 100 and the actual weights for the various treatments have been converted on this basis.

Plates 2 and 3 show the method of growing the plants, and also give a clear idea of the top and root growth in each case. The seedlings were grown for a period of about two weeks. Table 3 brings out a number of points of specific interest. It is especially significant that the 3-salt solution used as a check had in the beginning essentially the same pH value as the extract from 11A, but the latter (culture 1) gave a much poorer root growth than the check. The extract from 11B (culture 3), the soil of which has received the same ammonium sulfate treatment as 11A, but which has been limed at intervals of 5 years, had at the beginning, a pH value of 6.8 and gave a root growth of 114 as compared with 100 for the check. Again the extract from 11B plus

<sup>2</sup> 36 cc. KH<sub>2</sub>PO<sub>4</sub> M/2 }  
 10 cc. Ca(NO<sub>3</sub>)<sub>2</sub> M/2 } Made to 1 liter (1.75 atmospheres).  
 30 cc. MgSO<sub>4</sub> M/2 }

$\frac{1}{2}$  gm. of acid potassium phosphate (culture 7), with a pH value of 5.4, gave a root growth of 106.9, on the scale of 100 for the check, which is almost as much as the yield with the extract from 11B, unmodified, having a pH value of 6.8.

Extract 11A plus 13.5 cc. of lime water (culture 5) with a pH value of 6.8 (almost at the neutral point) gave a relative root growth of 83.8, which is distinctly less than the growth on culture 7 although the latter is decidedly more acid than the former. Nitrate of soda added to the extract depressed rather than increased plant growth.

These results would seem to indicate that acidity is not the only factor which has tended to hinder growth in this case.

#### *Culture-solution series 2*

The second series of culture solutions which was set up included extract from 11A and 11B and also from two heavily sulfured plots, one of which had subsequently been limed. Table 4 and plate 2 figure 2 show the arrangement and results.

TABLE 4  
*Series 2; Modified soil extract as a culture solution—reaction of solution and relative weights of crop*

CULTURE NUMBER		REACTION		RELATIVE WEIGHTS	
		Initial	Final	Tops	Roots
		pH	pH		
1	Shive's 3 salt solution ( $R_4C_2$ ).....	4.6	5.4	100.0	100.0
2	Extract from: Plot 11A.....	4.5	4.5	41.2	73.8
3	Plot 11B.....	7.2	7.0	36.1	112.5
4	Plot 11A + lime water.....	6.9	6.6	42.1	82.6
5	Plot 11A + lime water.....	8.4	7.0	43.7	98.8
6	Plot 11A + $NH_4OH$ and filtered to remove Fe and Al.....	6.7	5.0	42.3	43.1
7*	Plot 11A + $2\frac{1}{2}$ gm. acid phosphate.....	3.4	...	...	...
8*	Plot 11A + 5 gm. acid phosphate.....	3.2	...	...	...
9	Sulfur plot 5 limed.....	6.8	7.2	69.9	115.8
10	Sulfur plot 10 unlimed.....	4.6	4.6	30.4	59.6
11	Sulfur plot 10 + lime water.....	6.8	6.3	29.6	58.6
12	Sulfur plot 10 + 5 gm. acid phosphate.....	3.4	3.6	32.1	26.3

\* Plants in these cultures were a failure.

Here it will be noted that the 3-salt solution gave an excellent top and root growth notwithstanding the fact that the pH value was almost as low as that of extract 11A. Also extract 11B (culture 3) and extract 11A plus lime water (cultures 4 and 5), and sulfur plot 5 (culture 9) have all given excellent root growth.

Very poor root growth was secured with extract 11A (culture 2), sulfur plot 10 (culture 10) and sulfur plot 10 plus 5 gm. of acid phosphate (culture 12). In this last case the failure was no doubt as much due to the low pH value as to any other cause. In the case of culture 6 where ammonium hydroxide was added to extract 11A, it is quite possible the results would have been different if the solution had been boiled before it was filtered. Later work causes us to suspect that in this case we failed to remove all of the soluble aluminum salts, or that there was an excess of free ammonia.

The influence of the lime water in cultures 4 and 5 is noteworthy. This leads us to suggest that the calcium ion has a balancing effect on the aluminum even though the latter may not all be removed from the solution.

#### *Culture-solution series 3*

A third series was set up in which extracts from plots 11A and 11B were used in comparison with extracts from plots M (limed) and N (unlimed). For a number of years acid phosphate and muriate of potash have been used on plots M and N, but no ammonium sulfate or sulfur has been applied. No lime has been applied to plot N for a period of 15 years or longer, while plot M is limed at intervals of 5 years. These plots have been planted to soybeans continuously for a period of 10 years. Plot M produces each year an excellent crop while plot N has recently fallen to a yield of only a little more than a bushel per acre.

The arrangement of the series is indicated in table 5 and also plate 2, figure 3 and plate 3 figure 1.

In connection with this table it will be observed that extracts N and 11A (cultures 2 and 9) both gave a very low yield of tops and roots, but when these extracts were boiled with ammonium hydroxide and the precipitate removed and a small amount of iron and phosphoric acid added (cultures 5 and 11), each gave a total growth of roots and tops equal to or exceeding the growth made by the standard culture solution, notwithstanding the fact that the pH values were 5.2 and 5.1 respectively at the beginning and 4.6 and 4.0 respectively at the end. The yield of tops and roots, it will be noted, was about the same as the yield with the extract from plot M which began with a pH value of 7.0. It is thus quite clear that the ammonium hydroxide treatment puts out of action something that the mono-calcium phosphate (cultures 3 and 10) cannot counteract.

The response to the ammonium hydroxide treatment can hardly be attributed to the nitrogen, since nitrate of soda added to extract 11A in series 1 gave a lower yield than the unmodified extract, that is to say, nitrogen does not appear to be a limiting factor in the extract.

Potassium acid phosphate (cultures 7 and 13) lowered the pH value, but increased the yield over the untreated extracts in each case.

Ammonium acid phosphate lowered the pH value of both extracts, and in the case of plot N (culture 6) increased the yield over the untreated extract from this plot.

Cultures 14 and 15 constituting the second and third liter of leachings, respectively, from 11A, showed no root development and very poor tops. Culture 16 which represented the third liter of leachings from 11A and which was reinforced with 50 cc. of the 3-salt control solution gave very good root and top growth, though the weights of these are not reported in the table. It is possible that in this case the 50 cc. of control solution carried sufficient basic materials to counteract the toxic effect of the 11A extract.

TABLE 5

*Series 3; Modified soil extract as a culture-solution—reaction of solution and relative weights of crop*

CULTURE NUMBER		REACTION		RELATIVE WEIGHTS	
		Initial	Final	Tops	Roots
1	Shive's 3 salt solution ( $R_3C_3$ ).....	pH 4.6	pH 5.6	100.0	100.0
	Extracts from:				
2	Plot N.....	4.8	6.6	29.1	36.2
3	Plot N + 0.1 gm. $CaH_4(PO_4)_2$ .....	4.0	4.8	31.0	55.8
4	Plot N + lime water.....	7.0	6.6	25.5	50.1
5	Plot N + $NH_4OH$ , boiled and filtered, + small amount of Fe and $P_2O_5$ .....	5.2	4.6	72.8	114.2
6	Plot N + 0.1 gm. $NH_4H_2PO_4$ .....	4.4	4.4	36.4	49.8
7	Plot N + 0.1 gm. $KH_2PO_4$ .....	4.6	5.0	31.5	48.4
8	Plot M.....	7.0	6.9	73.0	122.6
9	Plot 11A.....	4.6	4.6	31.0	46.2
10	Plot 11A + 0.1 gm. $CaH_4(PO_4)_2$ .....	3.4	4.2	26.0	52.4
11	Plot 11A + $NH_4OH$ , boiled and filtered, + small amount Fe and $P_2O_5$ .....	5.1	4.0	126.6	88.3
12	Plot 11A + 0.1 gm. $NH_4H_2PO_4$ .....	3.7	4.4	25.0	35.5
13	Plot 11A + 0.1 gm. $KH_2PO_4$ .....	3.8	4.4	32.7	42.3
14	Plot 11A, 2nd liter of leachings.....	4.6	4.4	....	....
15	Plot 11A, 3rd liter of leachings.....	4.6	3.8	....	....
16	Plot 11A + 50 cc. Standard 3-salt solution.....	4.6	5.6	....	....
20	Plot 11B.....	7.2	6.4	41.1	120.0

It should be stated here that a liter of extract from plot N prepared as in the case of 11A yielded soluble iron and aluminum salts to the amount of 12 mgm. of  $Al_2O_3 \cdot Fe_2O_3$ , of which only a trace was iron. Plot M yielded no soluble iron and aluminum.

#### *Culture-solution series 4*

The fourth culture-solution was set up to further study the effect of modifying the extract from 11A. Barley was grown in this as in the previous series. The results are shown in table 6 and in plate 3, figures 2 and 3.

Here, as in the other series, the weights with the 3-salt solution were taken as 100 and the other weights calculated on this basis. An examination of the



table shows that cultures 2, 4, 5 and 6, all of which were the extract from 11A treated with ammonia to remove the aluminum compounds, gave very fair yields of both roots and tops. This is well shown in figure 2. In this connection it may be pointed out that in only one out of these four cultures was the pH value as high as 6.8 at the beginning, and that it was lower in all four at the end of the experiment than at the beginning.

TABLE 6  
*Series 4; Modified soil extract as a culture solution—reaction of solution and relative weights of crop*

CULTURE NUMBER		REACTION		RELATIVE WEIGHTS	
		Initial	Final	Tops	Roots
1	Shive's 3 salt solution ( $R_3C_2$ ) . . . . .	pH 4.6	pH 5.2	100.0	100.0
2	Extract from: Plot 11A (same as used in series 3) but treated with ammonia to remove Al and Fe . . . . .	4.8	3.7	73.2	48.9
3	Plot 11A + 0.1 gm. $CaH_2(PO_4)_2$ (same as used in No. 10 series 3) but treated with ammonia to remove Al and Fe . . . . .	5.0	4.7	36.2	25.5
4	Plot 11A with ammonia treatment to remove Al and Fe and a small amount of Fe added . . . . .	6.6	4.8	63.2	47.3
5	Plot 11A with ammonia treatment and a small amount of Fe and $P_2O_5$ added . . . . .	5.0	3.9	70.6	36.4
6	Plot 11A with ammonia treatment . . . . .	6.8	4.8	50.7	46.7
7	Plot 11A, evaporated to dryness, ignited and residue made to 200 cc. with distilled water . . . . .	7.2	6.0	24.5	63.2
8	Plot 11B treated as in No. 7 . . . . .	7.8	6.7	22.0	47.6
9	Plot 11A unmodified . . . . .	4.6	4.6	34.5	30.5
10	Same as No. 5 + 0.01 gm. $Al_2(SO_4)_3 \cdot 18H_2O$ . . . . .	4.6	4.3	62.5	46.6
11	Same as No. 5 + 0.025 gm. $Al_2(SO_4)_3 \cdot 18H_2O$ . . . . .	4.2	4.4	30.4	29.4
12	Same as No. 5 + 0.050 gm. $Al_2(SO_4)_3 \cdot 18H_2O$ . . . . .	3.5	3.8	20.2	31.7
13	Same as No. 5 + 0.100 gm. $Al_2(SO_4)_3 \cdot 18H_2O$ . . . . .	3.3	3.6	15.6	23.7
14	Same as No. 5 + 0.200 gm. $Al_2(SO_4)_3 \cdot 18H_2O$ . . . . .	3.1	3.4	18.4	20.5
20	Plot 11B unmodified . . . . .	6.8	6.4	28.5	51.8
21	Same as No. 5 + $H_2SO_4$ to make reaction pH 4.6 . . . . .	4.6	3.8	65.6	29.7
22	Same as No. 5 + $H_2SO_4$ to make reaction pH 4.2 . . . . .	4.2	4.0	48.9	36.8
23	Same as No. 5 + $H_2SO_4$ to make reaction pH 3.5 . . . . .	3.5	3.9	27.1	36.5

This lowering of the pH value in the presence of ammonium salts is in accord with recent work reported by Prince, Jones and Shive (8). They analyzed culture-solutions and concluded that the lowering of the pH values, when ammonium salts were present, was due, in all probability, to a difference in ion absorption by the plants, i.e., there was greater absorption of the ammonia ion than of the acid ion. Furthermore, in the absence of ammonium salts the reaction change in nutrient solutions in contact with the roots of

growing plants was towards the neutral point when a considerable amount of  $\text{Ca}(\text{NO}_3)_2$  was present. Prince, Jones and Shive (8) found that this change was directly correlated with high nitrate absorption. This idea is borne out by the reaction change of the standard culture solution, tables 4, 5 and 6.

In the case of culture 7, 200 cc. of the 11A extract was evaporated to dryness, ignited and the residue taken up with distilled water and used as a culture medium in the same way that the original extract was used. This, it will be observed, gave a very good root growth, though the plants were small.

The unmodified extract from 11A gave very poor root growth, the short stubby character of which is shown in culture 9.

Culture 10 with 0.01 gm. aluminum sulfate contained 1.53 mgm.  $\text{Al}_2\text{O}_3$ , whereas culture 9 contained by analysis 5.64 mgm.  $\text{Al}_2\text{O}_3$ . Comparing this with cultures 4 and 5 where 11A was treated with ammonia, it would appear that the small amount of alumina contained in culture 10 was not harmful to the plants, but when larger amounts were added, cultures 11-14 inclusive, the yield was reduced to less than the yield from the unmodified solution of 11A (culture 9). Since the increase in the amount of aluminum sulfate resulted in the lowering of the pH values, the injury may possibly be attributed partly to increased acidity and partly to increase in the amount of soluble aluminum.

Culture 10, which received the ammonia treatment (same as culture 5) with the addition of 0.01 gm. aluminum sulfate, gave a good growth of both roots and tops, but as the amount of aluminum sulfate was increased (cultures 11-14) the root and top growth was gradually depressed, the roots having the short stubby appearance observed in the unmodified extract from 11A. The pH value likewise decreased as the amount of aluminum sulfate was increased. It is significant that the results with 0.025 gm. and 0.05 gm. aluminum sulfate are quite similar to the results with the unmodified extract from 11A. The extract from 11B gave good results as indicated in culture 20.

Cultures 21, 22 and 23 were prepared by treating 11A extract with ammonia as in culture 5 and then adding dilute sulfuric acid to bring the pH values to 4.6, 4.2 and 3.5 respectively, thus giving pH values corresponding exactly with the pH values of cultures 10, 11 and 12. In the case of the former however, the plants were healthier in appearance than they were in the latter, and the sulfuric-acid treated cultures invariably gave larger yields (roots and tops) than the cultures which received the aluminum sulfate treatment. With the pH value down to 3.5 as in cultures 12 and 23, root development was seriously interfered with.

It is quite apparent from the results of this series that a culture solution may have a pH value as low as 4.6 and still give normal root development if the soluble aluminum has previously been removed by precipitation with ammonia. On the other hand a pH value of about 4.6 or lower with soluble aluminum salts present gives rise to a short stubby root growth, and usually depresses the top growth also.

## VEGETATION TESTS WITH ACID SOILS

*Series 1*

In this experiment barley was grown in glazed earthenware pots holding 3.5 pounds of soil from plot 11A, with special treatments as indicated in table 7.

The treatments were carried out in triplicate but for the purpose of condensing the report only the average for the 3 pots of each treatment is given. It will be remembered that this soil is distinctly acid having a pH value of about 4.6. At the close of the experiment a composite sample was made by sampling each of the 3 pots having the same treatment, and pH determinations were again made. These are reported in the table along with the dry weights of the crop.

TABLE 7  
*Series 1; Modified soil cultures—yield of dry matter and reaction of soil*

POT NUMBER	SPECIAL TREATMENT	DRY MATTER	FINAL REACTION
		gm.	pH
1	Nothing.....	0.0	4.7
2	10 gm. acid phosphate.....	5.2	4.5
3	20 gm. acid phosphate.....	6.3	4.6
4	Distilled water extract of acid phosphate.....	4.1	4.6
5	20 gm. ground limestone.....	9.0	7.5
6	20 gm. wood ashes.....	6.1	6.6
7	20 gm. basic slag.....	9.5	7.25
8	20 gm. Florida phosphate.....	4.4	5.3
9	5 gm. ammonium phosphate.....	2.8	4.9
10	5 gm. mono-calcium phosphate.....	5.7	4.7
11	1 gm. nitrate of soda.....	0.0	4.7
12	Watered with dilute solution calcium nitrate.....	0.0	4.7
13	Soil previously leached with distilled water.....	0.0	4.7
14	Soil roasted in furnace.....	4.1	5.6

The pots with untreated soil produced no barley,—only a small growth of crab grass. The pots that received acid phosphate gave a fair crop of barley but it should be noted that the applications of phosphate were exceedingly heavy, 20 gm. for 3½ pounds of soil being about equivalent to 25,000 pounds per acre. The crop was better with 20 gm. of acid phosphate than with 10 gm. The best yields, however, were obtained with ground limestone and with basic slag, and where these materials were used the pH values rose to 7.5 and 7.25, respectively. Wood ashes gave a yield almost as high as the 20 gm. of acid phosphate and raised the pH value of the soil to 6.6. Ground Florida phosphate rock and mono-calcium phosphate gave smaller yields than the 20-gm. application of acid phosphate. The Florida phosphate raised the pH value nearly 1 point, but the mono-calcium phosphate changed it only slightly.

Plants in the pots that received nitrate of soda and those that were watered with a solution of calcium nitrate were a failure, as were also those grown

in the leached soil. The roasted soil (heated so that organic matter was destroyed) showed an improvement as compared with the untreated soil. The roasting process, it will be observed, raised the pH value from 4.7 to 5.6, and it undoubtedly tended to convert soluble aluminum salts into the less harmful oxides.

These results are quite in accord with the culture solution work in showing that basic materials are most efficient in counteracting toxic substances contained in this soil. Two other series of pots were set up after this plan with soil from 11A, one of which was seeded to buckwheat and the other to rape, but the crops grew very slowly and were not harvested. It was quite evident, however, that had they been harvested and weighed the results would have corresponded in general to the results of the barley.

### Series 2

The soil used in this experiment was taken from the plot which had previously received a heavy application of sulfur and which became very acid (pH 4.4 to 4.5) due to the oxidation of the sulfur.

TABLE 8  
*Series 2; Modified soil cultures—yield of dry matter and reaction of soil*

POT NUMBER	SPECIAL TREATMENT	AVERAGE YIELD OF DRY BARLEY	FINAL REACTION
		gm.	pH
1-2	Check (untreated soil) . . . . .	Nothing	4.4
3-4	1 gm. acid phosphate . . . . .	Nothing	4.2
5-6	10 gm. acid phosphate . . . . .	3.15	4.25
7-8	50 gm. acid phosphate . . . . .	7.70	4.9
9-10	100 gm. acid phosphate . . . . .	6.25	5.3
11-12	1 gm. Mono-calcium phosphate . . . . .	2.55	4.4
13-14	2 gm. Mono-calcium phosphate . . . . .	3.80	4.5
15-16	1 gm. acid potassium phosphate . . . . .	4.30	4.5
17-18	2 gm. acid potassium phosphate . . . . .	4.55	4.5
19-20	20 gm. ground limestone . . . . .	8.90	7.2
21-22	Untreated soil from <i>limed, sulfur</i> plot (5a) . . . . .	7.95	7.4
23-24	20 gm. basic slag . . . . .	9.25	7.5

As in the other series 3.5 pounds of soil was used and special treatments, in duplicate, were given as indicated in table 8. Ten barley seeds were planted in each pot and the pots were kept at optimum moisture condition by the addition of water at intervals. Table 8 shows the average weights of barley at maturity and the pH value of the soil for the different treatments.

Germination was poor in the untreated soil and also in those soils that received acid phosphate. For the pots with untreated soil and those that received only 1 gm. of acid phosphate the crop was a failure. With 50 and 100 gm. of acid phosphate the crops were fair (see plate 4, fig. 1). The mono-calcium and potassium phosphates gave poor yields. Twenty grams of ground

limestone and 20 gm. of basic slag both gave good results, the basic slag showing the highest average as it did in series 1. The untreated soil from the *limed* sulfur plot also gave a good yield. This soil had been treated with sulfur at the same time the other soil was treated but about a year after the sulfur was applied it had been heavily limed, and at the time this experiment was started it had a pH value of a little above 7, which was about the same as the soil of the pots which received lime and basic slag.

It will be noted that in nearly all the other cases the pH value was below 5. It is significant that with a pH value of 4.9 pots 7 and 8 (table 8) gave an average yield of 7.7 gm. which is but little less than the yield with basic slag, having a pH value of 7.5.

### Series 3

The soil used in this third series of pots was an acid silt loam which had been without any lime or fertilizer treatment for many years. As in the preceding experiment, pots holding 3.5 pounds of soil were used. Special treat-

TABLE 9  
Series 3; Modified soil cultures—yield of dry matter and reaction of soil

POT NUMBER		AVERAGE YIELD OF DRY BARLEY	FINAL REACTION
		gm.	pH
1-2	Check (untreated soil) . . . . .	4.5	5.3
3-4	0.5 gm. $Al_2(SO_4)_3$ . . . . .	3.9	5.4
5-6	1 gm. $Al_2(SO_4)_3$ . . . . .	2.2	5.3
7-8	10 gm. Acid phosphate . . . . .	12.8	5.0
9-10	20 gm. Ground limestone . . . . .	7.4	7.6
11-12	20 gm. Basic slag . . . . .	13.4	7.3
13-14	20 gm. Florida rock phosphate . . . . .	11.4	6.1
15-16	20 gm. dibasic silicate . . . . .	12.2	8.0
17-18	1 gm. $Al_2(SO_4)_3$ + 10 gm. acid phosphate . . . . .	10.9	5.1
19-20	1 gm. $Al_2(SO_4)_3$ + 20 gm. ground limestone . . . . .	9.3	7.6
21-22	1 gm. $Al_2(SO_4)_3$ + 20 gm. basic slag . . . . .	12.6	7.3
23-24	1 gm. $Al_2(SO_4)_3$ + 20 Florida rock phosphate . . . . .	9.95	5.9
25-26	1 gm. $Al_2(SO_4)_3$ + 20 gm. dibasic silicate . . . . .	13.7	7.8
27-28	Check (untreated soil) . . . . .	5.12	5.4
29-30	0.5 gm. $Al(NO_3)_3$ . . . . .	3.00	5.3
31-32	1 gm. $Al(NO_3)_3$ . . . . .	1.60	5.3
33-34	0.5 gm. $Al(NO_3)_3$ + 10 gm. acid phosphate . . . . .	13.70	5.0
35-36	0.5 gm. $Al(NO_3)_3$ + 20 gm. ground limestone . . . . .	7.2	7.6

ments were given as indicated in table 9. The work was carried out in duplicate and 10 seeds of beardless barley were planted in each pot.

Germination was about 70 per cent in the untreated soil and 80 to 100 per cent for the various treatments. The table shows the dry weights of barley at maturity and the pH values of the soil representing the different treatments.

The yield with the untreated soil was small, and with 1 gm. of aluminum sulfate it was even less. With 10 gm. of acid phosphate (pots 7 and 8) it was

very much better, although the pH value of this soil, at the end of the experiment, was lower than that of the untreated soil (see plate 4, fig. 2 and table 9).

Additions of ground limestone, basic slag, Florida phosphate rock and dicalcic silicate all resulted in yields well above those obtained with the untreated soil. Also with these treatments the pH values were materially increased, dicalcic silicate raising it to pH 8. When, in addition to the acid phosphate and basic materials mentioned, 1 gm. of aluminum sulfate was applied (pots 17-26 inclusive) the yields were reduced only slightly or not at all as compared with the yields when the acid phosphate and basic materials were used without the aluminum sulfate. With 0.5 gm. and 1 gm. of aluminum nitrate the yields were even lower than when like quantities of aluminum sulfate were used. However with the addition of acid phosphate (pots 33 and 34) and ground limestone (pots 35 and 36) the yields were again raised, the former giving an average of 13.7 gm. and the latter 7.2 gm. It should be noted that in this case the acid phosphate treated soil, with a pH value of 5, gave a much better yield than the limestone treated soil with a pH value of 7.6.

From the data secured with pots 7-8, 17-18, and 33-34 it is quite evident that acidity is not the only factor which is responsible for low yields. These pots, having at the end of the experiment pH values of about 5, gave yields almost as large as any of the pots treated with basic materials and having pH values of 7 or above. It would appear, therefore, that the phosphoric acid has the power to put out of action some toxic material even though the soil remains distinctly acid. It must be borne in mind, however, that 10 gms. of acid phosphate for 3.5 pounds of soil is an unusually heavy application.

The low yields obtained with aluminum sulfate and aluminum nitrate, in a soil having higher pH values than the acid phosphate treated pots, point strongly to the aluminum as a disturbing factor. This is in accord with the results obtained in the culture solution work.

It would appear that aluminum compounds may be precipitated quite as readily by the use of heavy applications of soluble phosphates as by the use of ground limestone, or other basic material. It is indeed quite possible that some of the benefit which in the past has been attributed to acid phosphate as a source of phosphorus for plants, should be credited to its ability to precipitate, and thus put out of action, soluble aluminum compounds.

It is very certain that in many cases, especially among potato growers, it has been used in quantities many times as large as required by the crop, and apparently with good results. *Its ability to put out of action such toxic compounds and at the same time maintain the soil in a rather acid condition, may account for its great value as a potato fertiliser.*

#### SUMMARY

Soils which had become toxic through more or less abnormal treatment were leached with distilled water and the leachings analyzed for soluble iron and aluminum and other constituents, and were also used as culture media for the growing of plants.

In growing the plants, portions of the extract were used unmodified, and other portions were modified by the addition of soluble phosphates, lime water and by adding ammonia, boiling and filtering to remove soluble iron and aluminum.

Normal soils were also extracted and the extract used in comparison with the extract from the toxic soil. The analyses of the extracts from the good and poor soil brought out differences in soil reaction, water-holding capacity, total nitrogen and carbon and water-soluble iron and aluminum.

The culture-solution work makes it very clear that there is something in the extract from the toxic soil which interferes with root development of the plants. The extract from the normal soil gave normal root development, as did the standard culture-solution.

Additions of nitrate of soda and small amounts of soluble phosphates to the extract from the toxic soil, did not correct the unfavorable condition to an appreciable extent.

When the toxic extract was boiled with ammonium hydroxide, filtered and a trace of iron and phosphoric acid added to the filtered solution, normal root and top growth were obtained.

That this improvement was not due entirely to the neutralizing of the acidity is shown by the fact that the standard control solution, which gave normal root growth, had a pH value approximately the same as the pH value of the toxic extract. Aluminum sulfate when added to normal culture solutions in amounts equivalent to, and greater than the  $Al_2O_3$  present in the unmodified toxic extract, gave results similar to those with the unmodified extract. The pH values of the solutions were lowered by the addition of  $Al_2(SO_4)_3$ , but culture solutions brought to identically the same pH with  $H_2SO_4$ , produced plants much healthier in appearance than the cultures receiving the aluminum sulfate treatment. The results of the work point strongly to soluble aluminum as being responsible in part at least, for the unhealthy root development.

Vegetation tests carried out in small glazed earthenware pots gave results which confirm, in a large measure the culture-solution results. In the pot tests, however, heavy applications of acid phosphate to the toxic soil resulted in great improvement in growth even when the pH value was as low as 5. Small applications of soluble phosphates failed to give normal growth.

Heavy applications of ground limestone and basic slag gave good results in the pot experiments.

When aluminum compounds were introduced without a basic material such as lime, the yields were reduced to less than the yields with the untreated soil (check), but in the presence of liberal applications of basic materials, the aluminum sulfate and aluminum nitrate did not appreciably lower the yields of dry matter as compared with the yields from the basic materials without aluminum compounds.

The results of the pot work also lead one to suspect that soluble aluminum compounds constitute one of the causes of toxicity in soils.

The results of the work indicate that such toxicity may be largely overcome by heavy applications of soluble phosphates or by application of basic materials such as the different forms of lime and basic slag.

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## A METHOD FOR THE DETERMINATION OF "ACTIVE" ALUMINUM IN ACID SOILS<sup>1</sup>

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For many years the Rhode Island station has been studying the effects of "acid-soil" conditions, and more recently, of certain toxic substances resulting from or accompanying soil acidity, upon the growth of crop plants, and it has been shown that numerous plants are able to withstand fairly high concentrations of hydrogen ions in sand or water cultures, which are unable to produce any growth whatsoever in many soils of similar or even lower hydrogen-ion concentrations. One of the major reasons which has been advanced in explanation of these findings has been the presence of toxic concentrations of "active" aluminum salts in the acid, granitic soils under investigation (2, 3).

Sometime ago it became evident that a quantitative method of determining the actual concentrations of "active" aluminum in soils was necessary if further progress along these lines of study was to be made, and, with this end in view, a somewhat extended series of experiments was undertaken. Pure water, carbonated water, different concentrations of ammonium-chloride and nitrate solutions, and various normalities (1.0-0.01 *N*) of several of the more common organic and inorganic acids were all used in different proportions of soil to solvents, and shaken for different lengths of time. Aluminum determinations were made, using the solutions so obtained, and possible correlations between them and the growth of crop plants, known to be sensitive to "acid-soil" conditions were noted. Hydrogen-ion concentrations were also determined, using the soils employed and the solutions of the same, both before and after shaking. A full account of this work, together with other phases of the subject of aluminum toxicity to plants and its possible correction, will appear in a future publication from this station. The object of this brief paper is to describe the method finally adopted for the determination of "active" aluminum in soils, and to give reasons for its choice.

Neither pure water nor carbonated water dissolves sufficient quantities of aluminum, even from very acid soils, for accurate quantitative determination. The chief drawback to the use of a solution of a neutral salt, as ammonium chloride or ammonium nitrate, is that the soils are here subjected to the solvent action of resulting solutions of different hydrogen-ion concentrations;

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the more acid the soil, the greater the absorption, or at least removal, of the cation, which in turn results in a solution of much higher hydrogen-ion concentration, and with it, a solution of greater dissolving power for aluminum. Our experimental work has shown that, when a very acid soil is shaken with a normal solution of ammonium chloride (1 of soil to 5 of solvent), a solution of pH 2-3 results, whereas, if the soil used is neutral or slightly alkaline, the ammonium-chloride solution remains unchanged (practically neutral) after agitation. The Hopkins method for determining soil acidity is, of course, based upon this phenomenon of absorption or chemical reaction as the case may be. As it seemed wise to employ a solvent having the same dissolving power for soil aluminum *irrespective of soil reaction*, ammonium-chloride solutions were finally discarded although data of some value were obtained prior to this action.

The fact that solutions of acetic and citric acids are but slightly dissociated and, when partially neutralized, progressively ionize to maintain a constant percentage or concentration of hydrogen ions, suggested to us the choice of a solvent which would probably remain constant in composition and hence in dissolving power, irrespective of the reaction of the soils employed. Normal, 0.5 *N* and 0.05 *N* solutions of these acids were thus made and soils of various reactions shaken therein. The pH values of these acid suspensions after shaking, and the aluminum contents of the same after being passed through a porcelain pressure filter were determined. It was soon found that the normal solutions made too strong an attack upon the soils, resulting in too large amounts of aluminum being dissolved to permit of a proper correlation with the growth of "aluminum-sensitive" plants. The 0.05 *N* solutions, on the other hand, were unable to maintain a constant hydrogen-ion concentration when shaken with soils of unlike reactions. The 0.5 *N* solutions, however, being between the two in strength, permitted a correlation with plant growth, besides maintaining a fairly constant hydrogen-ion concentration when agitated with soils of widely different reactions. Table 1 presents data in proof of certain of these contentions as secured with acetic acid. Citric acid yielded similar results but, on account of subsequent analytical difficulties, was abandoned in favor of acetic acid. All of the soils reported upon came from the field plots of the Rhode Island Station (Miami silt loam), the dissimilarity in reaction being due to past, long-continued differences in fertilizer and lime treatments.

A solvent of proper strength having been secured, the next step was to find an accurate method for determining the small amounts of aluminum present in the acetic acid soil extracts, which also carried considerable quantities of calcium, magnesium, potassium, phosphate, sulfate and nitrate ions, besides traces of iron, manganese, silicon and other, less common substances. The aluminum-hydroxide method of Blum was first tried, but it was found impossible so to regulate the hydrogen-ion concentration as to prevent the co-precipitation of small amounts of calcium phosphate. Colorimetric

methods, using alizarin-3 and hematoxylin, also received attention but were discarded as hardly meeting the standard of accuracy desired. The aluminum-phosphate method of Carnot,<sup>2</sup> somewhat modified, was finally adopted and has given uniformly good results in the presence of considerable quantities of the substances above enumerated. It has been carefully tested out, using standardized solutions of aluminum sulfate, and has given theoretical results even in the presence of rather large amounts of calcium and phosphate ions.

The detailed method at present used in this laboratory for the determination of "active" aluminum in soils follows:

Shake the equivalent of 100 gms. of dry soil for 1 hour with 500 cc. (minus the volume of water in the soil) of 0.5 *N* acetic acid in an end-over-end shaking machine (8 r.p.m.); allow to stand a few hours or over night to settle, and then filter the supernatant liquid through

TABLE 1  
*Hydrogen-ion concentration of soils as affecting both the amounts of aluminum dissolved therefrom by different strengths of acetic acid and the hydrogen-ion concentrations of the suspensions after agitation*

REACTION OF SOIL USED	REACTION OF ACETIC ACID SUSPENSION AFTER AGITATION	Al <sub>2</sub> O <sub>3</sub> IN DRY SOIL
Normal acetic acid		
pH	pH	p.p.m.
4.5	2.6	1100
6.1	2.8	350
7.8	2.9	197
0.5 <i>N</i> acetic acid		
4.5	3.0	812
5.5	3.1	650
6.1	3.2	344
7.0	3.4	314
7.8	3.4	135
0.05 <i>N</i> acetic acid		
4.5	3.4	361
6.1	3.9	260
6.7	4.2	Trace
7.0	4.4	....

<sup>2</sup> Since this paper was written, Lundell and Knowles (4) have published results of a careful study of the several factors which influence the accuracy of this method. They give directions for precipitation and washing which are closely in accord with those which have been followed in our laboratory for the past year. The only points of difference between their method and ours are that they use macerated filter paper during the precipitation of the aluminum phosphate, and that, prior to the first precipitation, we boil with sodium thio-sulfate to reduce the traces of ferric iron usually present in soil extracts. The small amounts of titanium ordinarily present in soils are not soluble in 0.5 *N* acetic acid.

a Pasteur-Chamberland unglazed porcelain tube under pressure. Evaporate exactly 250 cc. of this filtrate to dryness (first on the hot plate and then on the water bath) after adding 10 cc. of aqua regia. Moisten the residue with 10 to 15 cc. of 1-to-1 hydrochloric acid and evaporate to dryness twice to dehydrate completely the silicon hydroxide. Finally digest the residue in about 100 cc. of hot, dilute hydrochloric acid and filter, washing the filter paper free from chlorides with hot water. To this filtrate containing the aluminum (which should measure about 200 cc.) add a decided excess of ammonium phosphate (5 cc. of a 10 per cent solution), and bring to a boil. Add 2 cc. of normal sodium thiosulfate solution and continue boiling for at least 10 minutes to reduce completely any ferric iron that may be present (ferrous phosphate is soluble in acetic acid). Just neutralize (use litmus paper) with  $\text{NH}_4\text{OH}$ , avoiding any excess, and immediately acidify very slightly\* with a few drops of a 50-per cent solution of acetic acid. Boil, let precipitate subside, and filter. Wash precipitate once with hot water and dissolve the aluminum phosphate from the free sulfur on the filter with hot, dilute (1 to 6) hydrochloric acid, refiltering if solution at first comes through turbid. Wash the filter with hot water until free from chlorids (about 200 cc.). Reprecipitate the aluminum phosphate by adding first to the hot filtrate 5 cc. of the 10 per cent ammonium-phosphate solution, then dilute  $\text{NH}_4\text{OH}$  until a *slight* permanent precipitate *just begins* to form, and then 10 cc. of a 15 per cent solution of ammonium acetate (the hot solution should test slightly acid with litmus paper at this point). Boil and filter as above; wash free from chlorides with hot water; ignite the  $\text{AlPO}_4$  to a constant weight. Multiply the weight of the  $\text{AlPO}_4$  precipitate, in grams, by 8370. This gives parts per million of  $\text{Al}_2\text{O}_3$  on the dry soil basis. A third precipitation may be necessary to separate completely the calcium and aluminum phosphates where the soils employed have received recently large dressings of either acid phosphate or lime.

Data have been obtained which show that air-drying acid soil increases the amounts of aluminum dissolved therefrom, by 15-20 per cent, hence it is advisable to use soil fresh from the field where possible.

Table 2 presents a few data selected from among many such, which show the existence of certain correlations between the reactions of the soils studied, the amounts of aluminum as determined by the above method, and the growth of plants known to be especially sensitive to "acid-soil" conditions. These crops were grown in Wagner pots in soils from differently treated field plots, and for the two crops here given, were all similarly fertilized in the pots, immediately prior to planting, with moderate applications of sodium nitrate, potassium sulfate and acid phosphate. The onion crop followed the let tuccrop in the same soils. The yields are averages from duplicate pots. This table shows a definite relationship between the amounts of "active" aluminum as here determined, soil reaction and crop growth. On the type of granitic soil here studied (over 80 per cent insoluble matter) and on others of similar composition and origin, data have been secured which show that, in the presence of 0.5 *N* acetic acid but irrespective of hydrogen ion concentration, soluble alumina in excess of approximately 225 p.p.m. of dry soil renders such soils somewhat toxic to crops which are especially sensitive to so-called "acid-soil" conditions.

In table 3 are tabulated a few results secured from three greenhouse beds of

\* If the solution at this point is made too strongly acid, a slight loss (about 30 p.p.m. of alumina) will result, as  $\text{AlPO}_4$  is slightly soluble in strong acetic-acid solutions (from 3 to 4 mgm. per 100 cc. of solvent).

lettuce. These beds are each approximately 0.0005 acre in area. The soil came originally from one of the acid experimental plots, but has since been heavily limed in each case. Besides lime, Bed 3 has received ordinary applications of acid phosphate, and Bed 6, heavy applications of well rotted compost. The yields given are for entire beds.

TABLE 2  
*Correlation of "active" aluminum, soil reaction and crop growth*

SOIL NUMBER	REACTION OF SOIL	"LIME REQUIRE- MENT" FOR 2,000,000 POUNDS OF SOIL*	Al <sub>2</sub> O <sub>3</sub> IN DRY SOIL	WEIGHT OF FRESH CROPS	
				Cos lettuce	Yellow globe onions
	pH	lbs. of CaO	p.p.m.	gm.	gm.
1	4.5	4410	812	0	0
3	4.7	3780	731	1	0
5	5.5	2520	650	24	0
6	6.1	1440	344	59	117
9	6.6	1170	290	77	197
10	7.0	720	314	78	256
14	7.8	450	135	127	240

\* Jones method as modified by the writer (1, p. 9).

It should be stated that, although Beds 3 and 4 had recently received a dressing of finely ground limestone equivalent to about 20 tons per acre, it had not completely reacted with the soil, as many small, white particles of calcium carbonate were readily discernible in the soil mass. These data—and they are substantiated by other of our experiments—would indicate that *lime alone* is unable to precipitate *entirely* all of the soluble aluminum present in the complex soil solutions of very acid soils when unaccompanied by fairly large applications of soluble phosphate.

TABLE 3  
*"Active" aluminum as influencing lettuce yields in greenhouse soils*

BED NUMBER	REACTION OF SOIL	Al <sub>2</sub> O <sub>3</sub> IN DRY SOIL	WEIGHT OF GREEN LETTUCE HEADS
	pH	p.p.m.	gm.
3	7.0	240	2270
4	7.0	82	5516
6	7.2	46	7105

In conclusion it should be stated that the data given in this paper are somewhat fragmentary, having been chosen from a considerable amount of similar material, and have been advanced simply to show the reasons for the adoption as well as the practical value of this method for determining "active" aluminum in agricultural soils.

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## THE EFFECT OF GYPSUM ON IOWA SOILS<sup>1</sup>

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Investigations dealing with the amount of sulfur in soils and crops and the loss of this constituent from soils, which have appeared in the literature in the last decade, have caused a renewed activity on the part of scientists to study the relation of sulfur to soil fertility.

Many analyses of soils have been made in various parts of the country and the sulfur supply has been found to be low in most cases. Crops remove considerable amounts of sulfates and there is apparently a large loss of the element in the drainage water. The importance of studying the sulfur problem and the relation of sulfur to soil fertility is evident. Since gypsum supplies this element in an available form it is only natural that the material should be considered with other sulfur fertilizers, and experiments are necessary to determine its actual value.

The present work was planned to determine first, the chemical and bacterial effects of gypsum on Iowa soils; and second, to study the effect of gypsum on crop growth and protein content of crops on various Iowa soils under different conditions.

### PART I. THE CHEMICAL AND BACTERIAL EFFECTS OF GYPSUM IN SOIL

#### THE EFFECT OF GYPSUM ON PHOSPHORUS AND POTASSIUM

Six 500-gm. portions of air-dry sieved soil of each of six important Iowa soil types were placed in 1-pint mason jars. Gypsum was added at the rate of 200 and 20,000 pounds per acre to duplicate jars and two of each type were untreated. Distilled water was added to bring the content up to the optimum. The jars were covered and kept at room temperature for seven months, water being added at frequent intervals to keep up the content. At the end of that time the soils were air-dried and analyzed for water-soluble phosphorus and potassium. The results of these analyses and the total phosphorus and potassium content of each soil are given in table 1.

The Shelby loam, having only 525 pounds of total phosphorus per acre, showed the largest amount of water-soluble phosphorus of any of the soils.

<sup>1</sup> Thesis without historical section and bibliography submitted to the faculty of the Iowa State College in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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With this soil the 200-pound gypsum treatment caused a slight increase in water-soluble phosphorus, whereas the 20,000 pound treatment decreased the solubility. With the Marshall silt loam the small application of gypsum increased slightly the solubility of the soil phosphorus while the heavy treatment had no effect whatever. Neither of the gypsum treatments exerted any effect on the phosphorus of the Carrington loam. The 200-pound gypsum

TABLE 1  
*Total and water-soluble phosphorus and potassium of six soil types treated with gypsum*

NUMBER	SOIL TYPE AND TREATMENT PER ACRE	PHOSPHORUS PER ACRE*		POTASSIUM PER ACRE*	
		Total	Water-soluble	Total	Water-soluble
	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>
	Shelby loam:				
1	Check.....	525	6.47	18,202	49.20
2	Gypsum 200.....	525	7.27	18,202	48.24
3	Gypsum 20,000.....	525	4.58	18,202	130.24
	Marshall silt loam:				
4	Check.....	1347	4.58	35,376	47.27
5	Gypsum 200.....	1347	5.65	35,376	33.76
6	Gypsum 20,000.....	1347	4.58	35,376	124.13
	Carrington loam:				
7	Check.....	1037	4.58	29,201	40.20
8	Gypsum 200.....	1037	4.58	29,201	43.09
9	Gypsum 20,000.....	1037	4.58	29,201	149.22
	Webster loam:				
10	Check.....	848	3.77	24,377	46.94
11	Gypsum 200.....	848	4.04	24,377	28.62
12	Gypsum 20,000.....	848	3.77	24,377	164.65
	Clinton silt loam:				
13	Check.....	956	3.77	31,131	54.67
14	Gypsum 200.....	956	4.04	31,131	35.69
15	Gypsum 20,000.....	956	4.04	31,131	111.59
	Tama silt loam:				
16	Check.....	1535	5.38	31,516	65.28
17	Gypsum 200.....	1535	7.27	31,516	60.78
18	Gypsum 20,000.....	1535	5.38	31,516	155.33

\* Calculated on the basis of 2,000,000 lbs. of soil.

treatment increased the water-soluble phosphorus of the Webster loam, but the difference was too small to be significant. The effects of the gypsum on the Clinton silt loam were likewise negligible. An increase of almost 2 pounds of soluble phosphorus was brought about by the small amount of gypsum on the Tama silt loam, but the larger treatment showed no effect.

Considering all the results, it is evident that the heavy application of gypsum had no effect on the content of water-soluble phosphorus of these soils while the 200-pound treatment gave a slight, though unmistakable, increase in all the soils studied except the Carrington loam.

The water-soluble potassium obtained from the 200-pound gypsum treatment was less in all the soils, except one, than that extracted from the untreated soils. The one exception was the Carrington loam. These results are exactly the opposite of those for water-soluble phosphorus.

The excessive gypsum treatment brought about a decided increase in the solubility of the potassium, in each soil type studied, the amount of potassium in the water extracts varying with the type of soil. There seems to be no question but that gypsum in large amounts may have a marked solvent action on the native soil potassium.

A second experiment to determine the effect of gypsum on phosphorus and potassium, was carried out on a neutral Carrington loam which had been used in a previous experiment to test the effect of gypsum on soil reaction. Gypsum had been added to this soil with and without  $\text{CaCO}_3$ , and the pots had been maintained at the optimum moisture-content for a period of five

TABLE 2  
*Total, water-soluble, and ammonium-citrate-soluble phosphorus and potassium per acre\**

TREATMENT OF NEUTRAL SOIL	TOTAL		WATER-SOLUBLE		AMMONIUM-CITRATE-SOLUBLE	
	Phosphorus	Potassium	Phosphorus	Potassium	Phosphorus	Potassium
	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
Check .....	1454	27,079	23.16	80.40	370.42	3069.28
500 lbs. gypsum .....	1454	27,079	19.39	109.98	404.10	1849.20
4000 lbs. calcium carbonate .....	1454	27,079	22.09	81.04	377.18	2934.60
4000 lbs. $\text{CaCO}_3$ plus 500 lbs. gypsum .....	1454	27,079	24.78	101.62	437.78	3344.64

\* Calculated on the basis of 2,000,000 lbs. of soil.

months. The soils were air-dried and analyzed for total, water-soluble and ammonium-citrate-soluble phosphorus and potassium. The results of these analyses are shown in table 2.

The gypsum when used alone seemed to cause a decrease in the solubility of phosphorus in water but when used with  $\text{CaCO}_3$  a slight increase was found. Lime alone had no effect on the phosphorus. Gypsum used with and without  $\text{CaCO}_3$  increased the amount of phosphorus dissolving in ammonium-citrate solution the larger amount being obtained when the  $\text{CaCO}_3$  was used. The  $\text{CaCO}_3$  treatment alone had no effect on the citrate-soluble phosphorus.

The 500-pound application of gypsum with or without lime caused a decided increase in the solubility of potassium while the  $\text{CaCO}_3$  alone had no effect.

The results for the ammonium-citrate soluble potassium were similar to those obtained for the water-soluble potassium, with the exception of the 500-pound gypsum treatment which decreased the soluble potassium.

To check the laboratory results by actual field tests, six square-rod plots were laid out in 1921 on a clover field on Carrington loam and treated with

different amounts of gypsum on May 9. Three months later a composite soil sample was taken from each plot and analyses were made for total, water-soluble, and ammonium-citrate-soluble phosphorus and potassium. The results are given in table 3.

The smallest application of gypsum effected a slight decrease in water-soluble phosphorus, while the 1000-pound gypsum treatment apparently caused a slight increase. The other treatments had no effect and it seems that gypsum did not have any influence on the water-soluble phosphorus in this soil. In the case of the ammonium-citrate-soluble phosphorus, the 200-pound gypsum treatment slightly increased the quantity of phosphorus going into solution, while the 1000 pounds of gypsum had no effect. The results of the 200 and 2000 pound gypsum treatments were not definite.

TABLE 3

*The effect of gypsum on phosphorus and potassium. 1-square-rod plots treated with different amounts of gypsum*

TREATMENT PER ACRE	TOTAL		WATER-SOLUBLE		CITRATE-SOLUBLE	
	Phosphorus	Potassium	Phosphorus	Potassium	Phosphorus	Potassium
	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
Nothing.....	1023	28,979	22.90	45.02	343.48	127.03
200 lbs. gypsum.....	1050	28,979	21.56	57.88	356.96	286.22
500 lbs. gypsum.....	1050	28,979	22.90	78.89	356.96	184.92
1000 lbs. gypsum.....	1050	28,979	24.24	91.65	343.48	220.29
2000 lbs. gypsum.....	996	28,979	22.90	184.92	323.28	247.63
Nothing.....	1023	28,979	22.90	41.80	343.48	144.72

Turning to the results for potassium it appears that each gypsum treatment effected a marked solubility in the soil potassium, the amount of potassium going into solution increasing with the larger additions of gypsum. The ammonium-citrate-soluble potassium determinations also show an unmistakable increase in the solubility of the soil potassium with gypsum. The increase was not proportional to the amount of gypsum added, however, the smallest treatment showing the greatest increase.

From these tests, it may be concluded that gypsum in small amounts may increase the solubility of the phosphorus and potassium in some soils. In others, no effects may appear. Excessive applications, however, have a large effect on the solubility of the soil potassium.

#### THE EFFECT OF GYPSUM ON NITROGEN

The effect of gypsum on the nitrogen transformations taking place in the soil was determined by measuring certain bacterial activities in a soil treated with different amounts of gypsum. Determinations were made at regular intervals for the ammonifying, nitrifying and azofying powers of the soil. For these experiments a neutral Carrington loam was selected.

Eighteen 4-gallon glazed earthenware pots were filled with 36 pounds of fresh soil containing 17 per cent moisture. Gypsum was applied to duplicate pots at the rate of 100, 200, 500, 1000, 1500, 2000, 5000 and 10,000 pounds per acre, thoroughly mixed with the soil and the moisture-content was then brought up to the optimum (23%) and kept at that point during the experiment.

The ammonifying power of the soils was determined in 100-gm. portions in tumblers with 5 gm. of dried blood. The incubation period was seven days and the ammonia was determined by the aeration method with  $\text{Na}_2\text{CO}_3$ . Nitrification was determined in air-dry soils also, with 100 mgm.  $(\text{NH}_4)_2\text{SO}_4$ . The incubation period was 4 weeks and the phenol-disulfonic-acid method was used to measure the nitrates.

Azofication was determined in a sterile dextrose solution, inoculating with infusions of fresh soil (1-cc. of infusion of 50 gm. soil to 100 cc. water, shaken 5 minutes). The nitrogen fixed was determined by the regular Kjeldahl method and the results are expressed as nitrogen fixed per gram of dextrose.

Two weeks after the experiment was started samples were drawn from each pot and tested for their ammonifying, nitrifying, and azofying power. The second and third samplings were made at two week intervals while the fourth, fifth and sixth samplings were taken at monthly intervals.

TABLE 4  
*Effect of gypsum on ammonification*

POT NUMBER	GYPSUM APPLIED	NH <sub>3</sub> PER 100 GM. AIR-DRY SOIL				
		1st sampling	2d sampling	3d sampling	4th sampling	5th sampling
	lbs.	mgm.	mgm.	mgm.	mgm.	mgm.
1, 2	Check	137.5	148.9	166.7	176.1	149.8
3, 4	100	127.6	156.6	171.4	172.9	155.3
5, 6	200	119.2	147.1	158.7	168.7	157.3
7, 8	500	118.4	142.8	155.6	151.1	134.7
9, 10	1,000	141.2	139.0	133.7	134.5	128.2
11, 12	1,500	142.5	139.5	135.5	141.7	132.8
13, 14	2,000	145.3	139.7	135.1	138.3	138.0
15, 16	5,000	145.1	144.3	121.5	137.4	126.5
17, 18	10,000	127.5	124.5	120.1	133.6	115.5

#### THE EFFECT OF GYPSUM ON AMMONIFICATION

The results of the tests for ammonification are shown in table 4.

At the first sampling, the 100-, 200-, 500-, and 10,000-pound treatments with gypsum depressed the ammonifying power of the soil, but the remaining treatments showed a slight increase in ammonia production. At the next three samplings in all cases except the 100-pound treatment, gypsum had a depressing effect on ammonification. The 100-pound gypsum application favored ammonification at the second and third samplings. At the fourth sampling the smallest treatment with gypsum gave a slight reduction in the amount of ammonia produced. Both the 100- and 200-pound gypsum treatments favored ammonification at the fifth sampling.

An average of the five samplings shows that the check produced 155.8 mgm. nitrogen; the 100 pounds of gypsum, 156.7 mgm.; and the 200-pound treatment, 150.2 mgm. as ammonia. It seems therefore that the 100- and 200-

pound applications of gypsum had no effect on the ammonifying power of this soil, and used at the rate of 500 to 10,000 pounds per acre it exerted an unfavorable effect on ammonification.

#### THE EFFECT OF GYPSUM ON NITRIFICATION

The nitrates present in the soils at the different samplings are shown in table 5.

There was considerable variation in nitrates present at the different samplings and therefore only the average of the five samplings will be discussed. The 2000- and 5000-pound gypsum treatments were the only ones which

TABLE 5  
*Nitrates in soils at various samplings*

POT NUMBER	GYPSUM APPLIED	NITRATE NITROGEN IN 100 GM. AIR-DRY SOIL					
		1st sampling	2d sampling	3d sampling	4th sampling	5th sampling	Average
	<i>lbs.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>
1, 2	Check	2.15	1.07	0.94	1.28	0.85	1.26
3, 4	100	2.23	1.19	1.03	1.55	0.95	1.39
5, 6	200	2.14	0.90	0.97	1.45	0.69	1.23
7, 8	500	1.70	1.06	1.01	1.22	0.70	1.14
9, 10	1,000	1.77	0.85	0.95	1.33	1.07	1.19
11, 12	1,500	1.88	0.92	1.01	1.25	1.14	1.24
13, 14	2,000	0.95	0.87	0.69	1.04	0.98	0.91
15, 16	5,000	0.66	0.88	0.74	1.08	1.06	0.88
17, 18	10,000	2.53	2.28	1.94	2.00	1.58	2.07

caused any appreciable decrease in the amount of nitrates present. The 100-pound application evidently favored nitrification in the soil although the increase was small. The largest application of gypsum, 10,000 pounds, showed a decided gain in nitrate nitrogen.

The results showing the effect of gypsum on nitrification appear in table 6.

At the first sampling, all of the gypsum treatments produced an unfavorable influence on the nitrification of ammonium sulfate, the depression being approximately the same for all treatments. At the second sampling the first four gypsum treatments were apparently favorable to the nitrifying bacteria for there was a decided gain in the nitrate nitrogen produced. The remaining treatments manifested a slightly harmful influence on nitrification.

At the third sampling, a slight decrease in nitrate nitrogen occurred with all the treatments except the 100-pound application. This was true also at the fourth sampling. At the fifth sampling, all of the soils produced practically the same amount of nitrate nitrogen.

Considering the general average of all the samplings, it is evident that gypsum when used in amounts from 100 to 1000 pounds per acre exerted no appreciable effect on the nitrification of ammonium sulfate, while larger applications of 1500, to 10,000 pounds per acre had a slightly depressing effect.

## THE EFFECT OF GYPSUM ON AZOFICATION

In table 7 appear the results for the azofication tests. Although the amounts of nitrogen fixed per gram of dextrose were exceedingly small the results for the different samplings agree very well. The largest amount of nitrogen was fixed by the untreated soil, in every case except at the second sampling. Except with the 100-pound application at this sampling, all of the gypsum treatments apparently decreased the amount of nitrogen fixed in the solutions. The amount of fixation decreased as the size of the gypsum treatment was increased and the 10,000-pound application almost inhibited azofication.

TABLE 6  
*The effect of gypsum on nitrification*

POT NUMBER	GYPSUM APPLIED	NITRATE NITROGEN IN 100 CM. OF AIR-DRY SOIL					
		1st sampling	2d sampling	3d sampling	4th sampling	5th sampling	General Average
	lbs.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
1, 2	Check	15.2	16.6	16.6	15.4	20.0	16.8
3, 4	100	13.0	18.6	16.6	15.4	20.0	16.7
5, 6	200	13.3	19.0	15.9	14.1	19.1	16.3
7, 8	500	13.6	18.2	14.7	14.1	18.3	15.8
9, 10	1,000	13.6	19.6	13.9	14.2	19.1	16.1
11, 12	1,500	14.0	15.4	14.2	13.7	17.4	14.9
13, 14	2,000	13.8	16.4	13.3	13.3	19.1	15.2
15, 16	5,000	12.9	13.6	14.1	14.9	19.1	14.9
17, 18	10,000	12.9	15.7	15.2	14.2	20.0	15.6

The samples taken at the fourth and fifth samplings were not tested for their azofying power. It was believed at the time that sufficient data had been secured to reach a definite conclusion on the effect of gypsum on azofication. It was thought, however, that the results might have been different had mannitol been used instead of dextrose and consequently at the sixth sampling, tests were made using mannitol instead of dextrose in the medium. The data given for the sixth sampling are very much the same as those obtained at the first three samplings. Dextrose, therefore, is apparently just as good as the more expensive mannitol for measuring the relative nitrogen-fixing power of variously treated soils.

## THE EFFECT OF GYPSUM ON ORGANIC MATTER

In order to determine the effect of gypsum on organic matter in the soil the following experiment was planned:

Three soils were obtained from different locations in the Wisconsin drift soil area, and all were classed as Carrington loam. The first soil had a lime requirement of 2260 pounds of  $\text{CaCO}_3$  per acre as determined by the modified Tacke method. The second soil was neutral in reaction and the third soil was highly basic, containing a large amount of calcium bicarbonate.

Twelve 500-gm. portions of each soil (air-dry) were weighed out in 800-cc. shaker-bottles, ground clover hay was added to each at the rate of 2 tons per acre and gypsum was applied in varying amounts as shown in table 8.

A layer ( $\frac{1}{4}$  inch thick) of  $\text{CO}_2$ -free fine gravel was first placed in the bottom of each bottle to permit even distribution of air through the soil. The moisture-content of the soils was adjusted to the optimum. The detailed arrangement of the carbon dioxide apparatus as described by Potter and Snyder, was followed in all its essential details. The system contained 24 bottles and likewise 24 carbon dioxide absorption towers, and only two soils were run at one time. During the early part of the experiment, titrations for carbon dioxide were made twice a week. Later once a week was all that was necessary. When the first two soils had ceased to evolve significant amounts of carbon dioxide, another series was started.

The total carbon dioxide production for each treatment for the entire period of experimentation is given in table 8; the individual results are not shown.

TABLE 7  
*The effect of gypsum on azofication*

POT NUMBER	GYPSUM APPLIED	NITROGEN FIXED PER GRAM OF DEXTROSE				
		1st sampling	2d sampling	3d sampling	6th sampling	General average
	lbs.	mgm.	mgm.	mgm.	mgm.	mgm.
1, 2	Check	4.4	1.7	2.1	4.0	3.06
3, 4	100	3.8	3.2	1.6	1.9	2.62
5, 6	200	3.2	2.1	1.4	2.6	2.32
7, 8	500	1.5	1.7	1.0	1.3	1.37
9, 10	1,000	0.9	1.3	1.1	1.3	1.15
11, 12	1,500	1.0	1.1	0.9	0.9	0.97
13, 14	2,000	0.7	1.7	1.0	1.5	1.22
15, 16	5,000	0.7	1.0	1.0	0.6	0.82
17, 18	10,000	0.5	1.4	0.8	0.1	0.70

The 100-pound gypsum treatment apparently had no effect on the carbon dioxide production in the acid soil. The 200-, 500-, and 1000-pound applications showed slight decreases in  $\text{CO}_2$  production but the differences were not large enough to be significant and the duplicates did not agree exactly. The 2000-pound treatment caused an increase in  $\text{CO}_2$  evolution, but the duplicates did not agree and one of them was almost the same as the check.

In the case of the neutral soil, the 100-, 200-, and 500-pound gypsum treatments showed practically the same amount of carbon dioxide production as the check soil.

The 1000-pound gypsum treatment was considerably higher than the first three treatments. The largest gypsum treatment showed a low production of  $\text{CO}_2$  but since one of the duplicates was lost no definite conclusion should be drawn.

With the basic soil, all of the treatments produced a decided increase in  $\text{CO}_2$  evolution with the exception of the 500-pound application and with this treatment there was no effect.

It is well known that the addition of  $\text{CaCO}_3$  to a soil high in organic matter causes a considerable increase in  $\text{CO}_2$  evolution. An experiment was run to determine the effect of gypsum on  $\text{CO}_2$  production when used with lime and organic matter. The acid soil used in the earlier test was employed for this work and the results are given in table 9.

TABLE 8  
*The effect of gypsum on carbon dioxide*

POT NUMBER	GYPSUM APPLIED	CARBON DIOXIDE PRODUCED		
		Acid soil	Neutral soil	Basic soil
	<i>lbs.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>
1, 2	Check	1595	1563	1579
3, 4	100	1605	1565	1663
5, 6	200	1517	1533	1618
7, 8	500	1564	1543	1554
9, 10	1000	1561	1639	1633
11, 12	2000	1671	1424	1690

Where no clover was added, the amount of  $\text{CO}_2$  produced was considerably less than for the clover treatment alone. Gypsum applied alone at the rate of 1000 pounds per acre decreased the amount of  $\text{CO}_2$  evolved and the soils receiving  $\text{CaCO}_3$  increased  $\text{CO}_2$  evolution to a marked degree. The 200- and 1000-pound applications of gypsum with the lime had no effect on the  $\text{CO}_2$  produced.

TABLE 9  
*The effect of gypsum and lime on carbon dioxide production*

NUMBER	TREATMENT	CARBON DIOXIDE PRODUCED
		<i>mgm.</i>
1, 2	Check.....	1155
3, 4	2 T. clover.....	1630
5, 6	2 T. clover and 2 T. lime.....	2008
7, 8	2 T. clover, 2 T. lime and 200 lbs. gypsum.....	1990
9, 10	2 T. clover, 2 T. lime and 1000 lbs. gypsum.....	2024
11, 12	1,000 lbs. gypsum.....	956

The work reported here would seem to warrant the conclusion that the use of gypsum in amounts employed in ordinary practice apparently had no effect on those types of bacteria that are active in breaking down the soil organic matter with the liberation of carbon dioxide. No effect was shown on the ammonifiers and nitrifiers and hence gypsum under the conditions of these experiments gave no indications of being a so-called "soil stimulant." Its indirect action on the soil must be attributed to chemical action rather than to an effect on bacterial activities.



## PART II. THE EFFECT OF GYPSUM ON CROP GROWTH AND PROTEIN CONTENT OF CROPS

In the spring of 1920, a number of field experiments were started to test the effect of gypsum on different crops when grown on various soil types in Iowa. A series of nine plots was laid out on fields in Story, Webster, Hardin and Wapello counties. The first three fields were located on Carrington loam, and the Wapello county field was on the Grundy silt loam. Gypsum was applied about May 1, 1920 to these plots at the rate of 200, 500 and 1000 pounds per acre with and without lime.

The plots were all  $\frac{1}{8}$  acre except those in Story county which were  $\frac{1}{4}$  acre in size. These fields were located near Ames, Fort Dodge, Eldora, and Farson. The first three fields mentioned were planted with oats, seeded to clover while the Farson field was planted to wheat, seeded to clover.

In a number of counties two  $\frac{1}{8}$ -acre plots were extended from a regular series of experimental plots belonging to the Soils Section of the Iowa Agricultural Experiment Station. This arrangement was made in Clinton, Scott, Lee, Buena Vista, Wapello and Van Buren counties. These fields are designated Delmar (Muscatine silt loam), Eldridge (Muscatine silt loam), Sawyer (Grundy silt loam), Truesdale (Carrington loam), Agency (Grundy silt loam) and Stockport (Grundy clay loam), respectively. The treatment for these two plots was 500 pounds of gypsum with and without limestone, except on the Agency and Stockport fields which received gypsum at the rate of 200 and 500 pounds per acre on clover in 1921.

In the spring of 1920 the Delmar and Eldridge fields were seeded to barley, and the Sawyer and Truesdale fields were seeded to oats. Four tons of ground limestone were applied with the gypsum treatment at the Truesdale field. Lime was applied to the Sawyer field after the oats were harvested.

The original plan of rotation called for each of these fields to be in clover in 1921. Unfortunately only three, Truesdale, Agency and Stockport, yielded clover in 1921. The Sawyer field was seeded to rye in 1921; the Delmar field was seeded to wheat; and the clover on the Eldridge field was plowed under early in the spring of 1921 for corn. No results for 1921 were obtained for these last two fields.

### THE EFFECT OF GYPSUM ON SMALL GRAINS

The results showing the effect of gypsum on oats at the Ames, Fort Dodge and Eldora fields are given in table 10. No records were obtained from the wheat plots at the Farson field.

On the Ames experimental field the gypsum treatments had practically no effect on the oats. Slight gains were noted in the case of the 500 and 1000 pound gypsum treatments with lime, but these were too small to be significant.

On the Fort Dodge field 200 pounds of gypsum gave an increase of 11.9 bushels of oats per acre over the average of the two check plots. The plot receiving lime alone was low and therefore no comparison can be made between it and the three plots receiving lime and gypsum.

On the Eldora field the gypsum caused a noticeable increase in oats. The 200 pound application gave a gain of 8.2 bushels of oats; 500 pounds, a gain of 4.8 bushels, while the plot receiving the large application of gypsum showed an increase of 15 bushels per acre when compared with the first check plot.

The plot with 500 pounds of gypsum and lime showed an increase of 10.9 bushels per acre over the plot receiving lime alone while the 200- and 1000-pound gypsum treatments with lime showed only small increases. The yields for the second check plot were rather high and made comparisons difficult.

TABLE 10  
*The effect of gypsum on yield of oats per acre*

PLOT NUMBER	TREATMENT	CARRINGTON LOAM		
		Ames	Fort Dodge	Eldora
		<i>bu.</i>	<i>bu.</i>	<i>bu.</i>
1	Check.....	44.2	52.4	48.3
2	200 lbs. gypsum.....	44.2	66.0	56.5
3	500 lbs. gypsum.....	46.6	56.5	53.1
4	1000 lbs. gypsum.....	46.3	51.0	63.3
5	200 lbs. gypsum and lime.....	44.5	57.8	57.8
6	500 lbs. gypsum and lime.....	46.6	57.8	65.3
7	1000 lbs. gypsum and lime.....	49.0	61.9	55.1
8	Lime.....	47.6	46.9	54.1
9	Check.....	44.2	55.8	61.9

The results showing the effect of gypsum on barley, oats and rye at the Delmar, Eldridge, Truesdale and Sawyer fields are given in table 11.

On the Delmar field one of the treatments with gypsum yielded a gain of 3.1 bushels of barley per acre while the duplicate plot gave no increase, hence no conclusions are possible. On the Eldridge field the two gypsum plots showed a slight increase in amount of barley produced.

TABLE 11  
*The effect of gypsum on yield per acre of barley, oats and rye*

PLOT NUMBER	TREATMENT	MUSCATINE SILT LOAM		CARRINGTON LOAM	GRUNDY SILT LOAM	
		Delmar (barley)	Eldridge (barley)	Truesdale (oats)	Sawyer (oats)	Sawyer (rye)
		<i>bu.</i>	<i>bu.</i>	<i>bu.</i>	<i>bu.</i>	<i>bu.</i>
1	Check.....	24.5	19.0	56.5	34.3	20.9
2	500 lbs. gypsum.....	24.5	23.6	56.1	50.9	31.5
3	500 lbs. gypsum.....	27.6	21.8	....	61.1	....
4	500 lbs. gypsum and lime.....	....	....	59.2	....	24.2

Gypsum used alone did not have any effect on the production of oats on the Truesdale field but with lime a small gain was secured.

On the Sawyer field gypsum effected a marked increase in the production of oats. The average increase for the two plots over the check plot was 21.7 bushels per acre. In 1921 on this same field gypsum alone increased the yield of rye by 10.6 bushels while with lime the increase was only 2.3 bushels.

## THE EFFECT OF GYPSUM ON RED CLOVER

The clover hay was harvested from each entire plot at Ames but on the other fields a strip was cut across each plot, measuring 1/30 of an acre, and the yields were calculated from this area.

The results of the 1921 red clover hay yields on the Ames, Fort Dodge, Eldora and Farson fields are found in table 12.

TABLE 12  
*The effect of gypsum on yield of red clover per acre*

PLOT NUMBER	TREATMENT	CARRINGTON LOAM			GRUNDY SILT LOAM
		Ames	Fort Dodge	Eldora	Farson
		lbs.	lbs.	lbs.	lbs.
1	Check.....	3000	2960	4620	4030
2	200 lbs. gypsum.....	3440	3220	4860	4070
3	500 lbs. gypsum.....	3472	3270	4470	4490
4	1000 lbs. gypsum.....	3840	3340	3390	4140
5	200 lbs. gypsum and lime.....	3760	2750	5400	5520
6	500 lbs. gypsum and lime.....	3140	2860	5460	5310
7	1000 lbs. gypsum and lime.....	3100	2750	6720	5140
8	Lime.....	2952	3120	5310	4520
9	Check.....	3260	4000	5970	4760

Gypsum was favorable to red clover on the Ames field. The 200-pound application increased the yield by 400 pounds. The 500-pound treatment produced only a slightly greater increase than the 200-pound application, but the plot receiving 1000 pounds of gypsum per acre increased the yield

TABLE 13  
*The effect of gypsum on yield of red clover per acre*

PLOT NUMBER	TREATMENT	CARRINGTON LOAM TRUESDALE	GRUNDY SILT LOAM AGENCY	GRUNDY CLAY LOAM STOCKPORT
		lbs.	lbs.	lbs.
1	Check.....	4268	4657	2272
2	200 lbs. gypsum.....	....	4830	2176
3	500 lbs. gypsum.....	4007	4554	2336
4	500 lbs. gypsum and lime.....	4181	....	....

840 pounds over the check. The plot receiving 200 pounds of gypsum plus lime showed a noticeable increase over the no-lime treatment, but poor yields were obtained on plots 6, 7, and 8, probably due to an attack of gophers in the early spring, and there was no evidence of any effect from the large amounts of gypsum plus lime.

Gypsum had practically no effect on the production of clover hay at the Fort Dodge field and at Eldora only the plot receiving 1000 pounds of gypsum and lime showed an increase in yield of clover hay. A gain of 1410 pounds per

acre of hay was secured on this plot when compared with the plot receiving lime alone and an increase of 750 pounds over the adjacent check.

On the Farson field lime and gypsum produced larger yields of clover hay than lime alone. These increases stand out prominently and amount to almost 1000 pounds of hay per acre. The 200-pound application of gypsum seemed to be more effective than the larger quantities. When gypsum was used without lime it had little effect on the production of clover hay.

The results of the tests with gypsum on clover on the Truesdale, Agency and Stockport fields are given in table 13.

Gypsum, when used either with or without lime, did not have any effect on the yield of clover hay on the Truesdale field. On the Agency and Stockport fields, gypsum was applied as a top dressing on the clover May 13 and 14, 1921, respectively, and the results indicate that the treatments were made apparently too late to permit the gypsum to have any effect.

TABLE 14  
*The effect of gypsum on alfalfa—Carrington loam*

PLOT NUMBER	TREATMENT	YIELD PER ACRE				AVERAGE N IN 2d AND 3d CUTTING
		1st cutting	2d cutting	3d cutting	Total	
		lbs.	lbs.	lbs.	tons	per cent
1	Check.....	1820	3856	5200	5.43	2.46
2	200 lbs. gypsum.....	2284	4164	5392	5.92	2.45
3	500 lbs. gypsum.....	2108	3868	5568	5.77	2.42

#### THE EFFECT OF GYPSUM ON ALFALFA

Two 1/40-acre plots were laid out in an alfalfa field on the Agronomy farm of the Iowa Experiment Station in the spring of 1921. They were top-dressed with gypsum on April 14, the first plot at the rate of 200 pounds and the second at the rate of 500 pounds of gypsum per acre.

The results for the three cuttings as well as the average per cent of nitrogen found in the alfalfa from the second and third cuttings are given in table 14.

It will be noted that the gypsum treatments increased the yield of alfalfa hay at each cutting but the effect was most pronounced at the first cutting. The 200-pound application increased the yield of this cutting 464 pounds. At the second cutting the same treatment increased the yield 308 pounds, and at the third cutting an increase of only 192 pounds was secured. The yields from the plot receiving 500 pounds of gypsum per acre were not quite as large as those obtained with the smaller applications.

The total yield for the three cuttings showed an increase for the 200-pound gypsum treatment of 964 pounds of hay while for the 500-pound application the increase was 668 pounds.

The analyses for total nitrogen on the second and third cuttings show that gypsum did not affect the nitrogen content of the alfalfa hay.

## THE EFFECT OF GYPSUM ON PROTEIN CONTENT OF OATS AND CLOVER

Analyses for total nitrogen were made on samples of oats from the field plots at Ames, Fort Dodge, and Eldora, and also on samples of clover hay from the plots at Ames. The results of these analyses and the per cent of crude protein in each sample are given in table 15.

From the analyses of the clover hay it may be seen that there was a slight decrease in the total nitrogen in the clover on the plots receiving gypsum and lime. This is also true for the 1000-pound gypsum treatment. These differences, however, were too small to be of significance.

TABLE 15  
*Effect of gypsum on protein content of clover and oats*

PLOT NUMBER	TREATMENT PER ACRE	AMES				FORT DODGE		ELDORA	
		Clover		Oats—crude protein		Oats—crude protein		Oats—crude protein	
		Total nitrogen	Crude protein	Grain	Straw	Grain	Straw	Grain	Straw
		per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
1	Check .....	1.97	12.31	11.03	2.27	10.65	1.92	13.83	4.37
2	200 lbs. gypsum .....	1.98	12.37	Lost	Lost	10.65	1.66	13.30	3.23
3	500 lbs. gypsum .....	1.93	12.06	10.06	1.66	10.65	2.10	12.15	2.88
4	1000 lbs. gypsum .....	1.84	11.50	11.68	1.57	10.65	1.66	11.71	2.88
5	200 lbs. gypsum and lime .....	1.70	10.62	10.59	2.36	11.11	2.62	11.89	2.88
6	500 lbs. gypsum and lime .....	1.87	11.68	10.40	1.92	11.29	1.92	12.16	2.62
7	1000 lbs. gypsum and lime .....	1.76	11.00	10.15	1.48	11.29	2.62	13.39	2.97
8	Lime .....	1.89	11.81	10.40	1.66	11.29	.....	11.64	2.53
9	Check .....	1.94	12.12	10.59	1.75	11.55	1.83	11.64	2.80

The data presented for the percent of crude protein in the oat grain and straw show that gypsum did not have any marked effect on the total nitrogen content of this crop. The check plots on the Ames and Eldora fields did not agree and hence definite conclusions cannot be drawn.

## THE EFFECT OF GYPSUM ON RED CLOVER

*Greenhouse Experiment*

To check up the data obtained in the field, an experiment was planned to test the effect of gypsum on clover grown in pots in the greenhouse.

The soil obtained for this work was the same as that used in the field test at Ames. It was classed as Carrington loam and had a lime requirement of two tons of  $\text{CaCO}_3$  per acre. Sixteen 4-gallon stoneware pots were half filled with the subsurface soil, and then the surface soil was added making a total of 35 pounds of soil (air-dry) for each pot. The same treatments used

on the full series of field plots were made in duplicate in this experiment. The limestone was thoroughly mixed with the surface soils, the pots were weighed and water was added to give optimum moisture conditions. All the pots were then seeded to red clover. When the plants were large enough they were thinned to 5 plants per pot and the gypsum treatments were made as a top-dressing and worked into the soil.

The crop was harvested when the majority of the plants were in full bloom and the results obtained from the experiments are given in table 16.

It is rather difficult to draw any definite conclusions from the results. The 200-pound application of gypsum had no effect on the yield of clover. The 500-pound treatment on the other hand showed a noticeable increase. The 1000-pound gypsum treatment seems to have had a depressing effect on the yield of the clover. The pots receiving 200 and 1000 pounds of gypsum with lime showed distinct gains in yields over the pots with lime alone. The

TABLE 16  
*The effect of gypsum on yield and nitrogen content of clover in greenhouse experiment*

POT NUMBER	TREATMENT	YIELD PER POT— DRY WEIGHT	AVERAGE N CONTENT PER POT
		<i>gms.</i>	<i>per cent</i>
1, 2	Check.....	19.5	1.69
3, 4	200 lbs. gypsum.....	18.5	1.89
5, 6	500 lbs. gypsum.....	22.0	1.78
7, 8	1000 lbs. gypsum.....	15.0	2.10
9, 10	200 lbs. gypsum and lime.....	26.0	1.87
11, 12	500 lbs. gypsum and lime.....	23.0	1.96
13, 14	1000 lbs. gypsum and lime.....	28.5	1.96
15, 16	4 tons lime.....	24.0	1.83

500-pound gypsum and lime application showed a slight decrease in yield from the lime alone.

Slight increases in total nitrogen content of the clover hay were observed in every case where gypsum was added to the soil but the differences were too small to warrant a definite conclusion.

It is evident from these experiments that yields of oats, barley, rye and clover may be increased on some soils and not on others. Alfalfa yields were greater when gypsum was used. The protein content of the various crops, however, was not influenced to any striking extent.

#### THE EFFECT OF GYPSUM ON SOIL REACTION

At the time the oats crops were harvested from the Ames, Fort Dodge and Eldora fields, samples of soil were taken from each plot and tested for lime requirement and hydrogen-ion concentration, in order to note the effect of gypsum on soil acidity under actual field conditions. The samples were taken about 4 months from the time the gypsum applications were made. The results of these tests are shown in table 17.

Examining the pH values for the Ames plots we note that the gypsum treatments had practically no effect on the hydrogen-ion concentration of these soils. The slight increase in acidity (pH 0.11) observed from the 1000-pound application was probably too small to be considered important. The pH values for the Fort Dodge plots also showed that gypsum had no effect whatever on the hydrogen-ion concentration of the soils. A slight increase in acidity (pH 0.2) was noted on the 1000-pound gypsum plot of Eldora field. The 500-pound gypsum application slightly decreased the acidity.

The lime requirement tests checked fairly well in showing that the use of gypsum had little or no effect on the acidity of the soil, as determined by the modified Tacke lime requirement method. From these tests it seems evident

TABLE 17  
*Effect of gypsum on soil reaction*

PLOT NUMBER	TREATMENT (POUNDS PER ACRE)	HYDROGEN-ION CONCENTRATION			LIME REQUIREMENT PER ACRE BY MODIFIED TACKE METHOD		
		Ames	Fort Dodge	Eldora	Ames	Fort Dodge	Eldora
		pH	pH	pH	lbs.	lbs.	lbs.
1	Check.....	6.01	5.39	5.52	4200	7920	4480
2	200 lbs. gypsum.....	6.01	5.35	5.52	4000	8200	5040
3	500 lbs. gypsum.....	5.95	5.37	5.69	4440	6800	4000
4	1000 lbs. gypsum.....	5.90	5.35	5.32	3880	7120	4400
5	200 lbs. gypsum and lime.....	6.23	6.01	6.54	3880	4160	2080
6	500 lbs. gypsum and lime.....	6.35	7.13	6.17	3520	760	1600
7	1000 lbs. gypsum and lime.....	6.35	5.85	6.37	3060	3360	840
8	Lime.....	6.37	5.69	6.37	3300	5000	720
9	Check.....	6.44	5.35	5.52	3640	7080	4600

that gypsum in amounts used in ordinary practice did not have any effect on the lime requirement of the soils. When applied at the rate of 200 or 500 pounds per acre it did not raise or lower the hydrogen-ion concentration of the soil as measured by the hydrogen electrode but applications of 1000 pounds caused slight increases in hydrogen-ion concentration on the plots at Ames and Eldora.

#### THE SULFUR CONTENT OF RAINWATER

During the years 1920-1921 samples of rain and snow were collected on the Agronomy farm of the Iowa Agricultural Experiment Station which is two miles south of Ames. A 4-gallon glazed stoneware crock was used. The samples were stored in bottles sealed with paraffined glass stoppers. The analyses for eighteen months from November 1, 1921 to April 30, 1922 are given in table 18. A total rainfall of 38.17 inches was recorded. The total amount of sulfur added to the soil in the rain and snow for the entire period was 18.34 pounds, or the equivalent of 45.77 pounds of  $\text{SO}_2$  per acre.

By comparing the monthly data for sulfur content it may be seen that generally the largest amount of sulfur was brought to the soil during the growing season from March to October when conditions were the most favorable for the bacteria which bring about the decomposition of organic matter with the liberation of hydrogen sulfide. The amount of sulfur found in the rainwater for the spring, summer and fall months was fairly constant, with the exception of July, when only 0.40 pounds of sulfur per acre was obtained. This low figure was probably due to the fact that this month was too dry for vigorous bacterial action.

TABLE 18  
*Rainfall and sulfur content of rain and snow November 1, 1920, to April 30, 1922*

MONTH	RAINFALL		
	Amount	SO <sub>2</sub> content	S content
1920	in,	lbs. per acre	lbs. per acre
November.....	1.08	0.74	0.30
December.....	0.58	0.84	0.34
1921			
January-February.....	1.00	5.04	2.02
March.....	1.28	3.74	1.50
April.....	2.91	5.49	2.20
May.....	2.83	4.57	1.83
June.....	4.77	2.67	1.07
July.....	1.11	1.00	0.40
August.....	7.35	5.47	2.19
September.....	6.76	3.77	1.51
October.....	1.25	3.67	1.47
November-December.....	1.12	1.75	0.70
1922			
January-February.....	1.93	2.32	0.93
March.....	1.50	1.53	0.61
April.....	2.70	3.17	1.27
Total.....	38.17	45.77	18.34

Other investigators have shown that the amount of sulfur added to the soil in the rainwater does not vary greatly from year to year. A total of 14.89 pounds of sulfur was added to an acre of soil during the year 1921 and the annual precipitation was about average. It may be concluded therefore that from twelve to sixteen pounds of sulfur per acre may be regarded as the average amount of sulfur which is added to the soil by the rainwater under actual farm conditions in Iowa.



## THE AMOUNT OF SULFUR IN DRAINAGE WATER

A study of the loss of sulfur in drainage water was made during the year 1921-22. A 4-gallon glazed stoneware pot was placed on a small platform. The small drainage opening near the bottom of the pot was fitted with a small metal spigot, the outlet of which entered a smaller glazed stoneware pot through an improvised galvanized cover. The cover was arranged to exclude particles of dust as well as rainwater.

A layer one inch thick of carefully washed sulfur-free sand was placed in the bottom of the upper pot. Fresh Carrington loam surface soil was then added making a total depth of six inches of soil which included a good clover sod.

TABLE 19  
*Loss of sulfur in drainage water*

MONTH	DRAINAGE WATER	WEIGHT $\text{BaSO}_4$ PER 250 CC. DRAINAGE WATER	RELATION OF S LOST TO TOTAL DRAINAGE WATER
1921	cc.	gm.	per cent
April.....	225	0.0389	0.00213
August.....	1378	0.0377	0.00206
September.....	5870	0.0315	0.00172
1922			
February.....	265	0.0404	0.00221
March.....	1000	0.0322	0.00176
April.....	2450	0.0414	0.00226
Average.....			0.00202

During the year 1921, the rainfall was so scattered over each month that drainage water from the soil in the pot was collected only during the months of April, August, and September. In 1922 drainage water was obtained during the months of February, March, and April. The results secured for these analyses are given in table 19.

Although the amount of drainage water obtained during the months noted in the table varied greatly, there was a striking uniformity in the analyses for total sulfur lost and the per cent lost was almost constant.

Sulfur averaged .00202 per cent of the drainage water from this soil. One inch of rain per acre weighs 226,000 pounds. From a study of a number of investigations it seems that 50 per cent of the total rainfall is lost in the drainage water. Using these figures, the calculated loss of sulfur from a 30-inch rainfall on the Carrington loam would be 67.5 pounds per acre per year.

The amount of sulfur lost in the drainage was over four times as great as that added to the soil by the rain and snow. While this applies only to the Carrington loam, the sulfur lost from other types of soil is probably quite as

great. It is quite apparent that the continued loss of sulfur at this rate will lead to a rapid depletion of the native sulfur supply of the soil.

#### GENERAL CONCLUSIONS

Some important conclusions may be drawn from this work. In the first place it is quite apparent from both the chemical data and field work that the soil type plays a prominent part in the effects of gypsum on soils. With certain soils gypsum in the amounts usually employed in agricultural practice affected the soil phosphorus and the soil potassium, rendering both of these constituents more soluble in a water-extract. With other soils little or no effect was observed. When gypsum was used in excessive quantities, however, there was a marked increase in the amount of water-soluble potassium in all the soils studied.

The smaller applications of gypsum did not have any effect on ammonification and nitrification, while the larger amounts were slightly unfavorable to these bacterial processes. All of the gypsum treatments were unfavorable to azofication as measured by the amount of nitrogen fixed in solution per gram of dextrose.

The results on carbon dioxide production show that gypsum did not hasten the decomposition of the soil organic matter except perhaps, in a highly basic soil.

In the field experiments, gypsum proved favorable on the clover and small grain crops in several instances. Possibly the effects on clover would have been more pronounced had the applications been made as top-dressings directly to the clover instead of to the grain crops.

Gypsum at the rate of 200 pounds per acre exerted a distinctly beneficial effect on the production of alfalfa hay. It was unfortunate that the results of the experiments on alfalfa were limited to one field, because it is believed that greater increases from the use of gypsum would be noted on alfalfa than on other crops.

Finally the analyses of rain and drainage waters emphasize further the importance of the sulfur problem in agriculture. The amount of sulfur lost in the drainage is far greater than that added to the soil in the rainwater. It is quite evident that even though soils may be well supplied with sulfur at the present time this sulfur is constantly being oxidized to the sulfate in which form it is readily leached away in the drainage waters. If any system of soil fertility is to be permanent, a provision must be made for the addition of sulfur in some form to the soil. This may be accomplished economically through the use of barnyard manure, gypsum or acid phosphate which contains about 60 per cent of gypsum.



## ADSORPTION AND REPLACEMENT OF PLANT FOOD IN COLLOIDAL OXIDES OF IRON AND ALUMINUM

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### INTRODUCTION

The work by Wiley and Gordon (1) on colloidal silica and by Starkey and Gordon (2) on different soil colloids under a varying hydrogen-ion concentration, made it of interest to make a more extensive investigation with the colloidal oxides of iron and aluminum. It was first purposed to prepare colloidal oxides of iron and aluminum so that they would obtain nothing other than a trace of the chloride, and find their maximum adsorption from some of the solutions that are commonly used in plant nutrition work; and secondly, to find the necessary conditions for the replacement of this plant food.

### MATERIAL USED

Ferric and aluminum hydrogels were prepared by treating hot solutions of ferric chloride and aluminum chloride, respectively, with ammonium hydroxide and washing the resulting hydrogels, with distilled water until there was no test for ammonia or chloride. The hydrogels containing about 96 per cent water were then placed in a glass-stoppered jar. The gels were prepared in sufficient quantities so that one preparation answered for all the subsequent experiments.

Only the hydrogel was used in this work for it was believed that any salt that would allow a hydrosol to exist in the soil would produce a peptization of the hydrogel. The amount of peptization varied with the different salts used, but was very highly marked in the case of the hydrogel of iron when treated with potassium acid phosphate.

The salts were the same as those used with colloidal silica; namely, calcium acid phosphate, calcium sulfate, calcium nitrate, magnesium acid phosphate, magnesium sulfate, magnesium nitrate, potassium acid phosphate, potassium sulfate, and potassium nitrate.

### METHODS

Approximately 0.1 *N*, .05 *N* and .025 *N* solutions of the above named salts were prepared by weighing out the calculated amounts of the salts and then determining the exact concentrations. The calcium was determined by precipitating with ammonium oxalate in a solution slightly acid with acetic acid, washing the precipitate, dissolving it in sulfuric acid, and then titrating the resulting solution with potassium permanganate. The magnesium was determined as  $Mg_2P_2O_7$ , and the potassium was determined as  $K_4PtCl_6$ . Phosphates were determined by precipitating in nitric acid solution with ammonium molybdate, and titrating with

standard sodium hydroxide. Sulfates were determined as  $\text{BaSO}_4$ . Nitrates were reduced with powdered iron and sulfuric acid, distilled with sodium hydroxide, and measured by titrating the ammonia.

In the determinations, 400 cc. of the respective solutions were shaken with definite portions of the hydrogels until equilibrium was established. The amount of the iron hydrogel used was that which would correspond to three grams of the gel when it had been dried to a constant weight at  $110^\circ\text{C}$ ., while in the case of the aluminum only enough hydrogel was added to correspond to one gram of the dry material. The water added with the hydrogel was figured as water of dilution in calculating the original concentration of the solution. In most cases the mixtures were thoroughly shaken by hand for one minute at intervals of one hour. At the end of the first day, and at subsequent intervals as indicated in the table 2, aliquots of the solutions were taken and their concentrations determined. The shaking was continued until there was no further change in concentration. The total change in concentration gave the total adsorption.

### RESULTS

Table 1 shows that there was only a slight adsorption of nitrates in the order of calcium, magnesium, and potassium. With the aluminum hydrogel, no

TABLE 1  
*Adsorption of nitrates and sulfates by the colloidal oxides of iron and aluminum*

SALT USED	METAL ADSORBED BY 1 GM. GEL			NEGATIVE ION ADSORBED BY 1 GM. GEL		
	0.025 N	0.05 N	0.1 N	0.025 N	0.05 N	0.1 N
<i>Iron hydrogel</i>						
	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
$\text{KNO}_3$ .....	None	None	None	None	None	None
$\text{Mg}(\text{NO}_3)_2$ .....	1.7	2.2	3.4	6.0	7.0	19.0
$\text{Ca}(\text{NO}_3)_2$ .....	2.2	3.4	8.4	6.6	8.4	21.7
$\text{K}_2\text{SO}_4$ .....	2.1	3.5	4.0	3.4	4.3	5.9
$\text{MgSO}_4$ .....	4.8	5.8	7.1	19.3	23.2	28.3
$\text{CaSO}_4$ .....	15.0			35.3		
<i>Aluminum hydrogel</i>						
	3.7	7.5	9.2	4.4	8.4	12.1
$\text{K}_2\text{SO}_4$ .....						
$\text{MgSO}_4$ .....	6.1	8.7	14.6	29.6	41.4	65.9
$\text{CaSO}_4$ .....	19.2			46.0		

adsorption of the nitrates of these three metals could be observed. Where adsorption takes place in either gel, approximately equivalent amounts of the ions of the same salt were adsorbed. The small solubility of calcium sulfate has limited its use to one concentration, but even then the amount of adsorption was greater than in the more concentrated solutions of potassium and magnesium sulfates. The adsorption of potassium and magnesium sulfates increased with an increase in concentration. The results with the sulfates indicates that the order of adsorption is calcium, magnesium, potassium.

Experiments were next tried where phosphate solutions were used instead of sulfate. Here the adsorption was found to be much greater, and it took much

TABLE 2  
*Adsorption of phosphates by colloidal ferric oxide*

SALT USED	TIME	METAL ADSORBED BY 1 GM. GEL			PO <sub>4</sub> ADSORBED BY 1 GM. GEL		
		0.025 N	0.05 N	0.1 N	0.025 N	0.05 N	0.1 N
	days	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
KH <sub>2</sub> PO <sub>4</sub> .....	1	21.9	51.7	88.0	121.9	214.4	311.5
	3	27.9	62.3	99.7	133.3	240.8	344.4
	7	37.5	69.3	103.7	141.7	255.5	380.8
	14	40.6	70.5	107.2	143.4	265.4	411.1
	21	40.6	70.5	107.2	143.4	265.4	411.1
MgH <sub>2</sub> (PO <sub>4</sub> ) <sub>2</sub> .....	1	19.3	31.8	37.6	143.8	242.9	294.0
	7	20.4	33.4	42.5	153.0	265.0	332.2
	14	21.0	34.3	46.7	158.0	275.3	358.6
	21	21.5	36.3	47.4	162.1	281.5	367.5
	28	21.5	36.3	47.5	162.1	281.5	367.5
CaH <sub>4</sub> (PO <sub>4</sub> ) <sub>2</sub> .....	1	43.0	61.4	82.7	205.8	302.7	402.1
	3	46.5	69.8	100.6	231.3	345.2	485.0
	7	48.9	71.4	103.3	242.7	377.4	550.3
	14	51.3	78.8	113.7	259.4	402.6	574.6
	21	54.3	79.8	116.9	266.6	408.4	586.2
	28	54.5	82.4	120.4	266.6	411.3	597.9
	35	54.5	83.3	121.6	266.6	421.6	607.2
	42	54.5	83.3	121.4	266.6	421.6	609.9

TABLE 3  
*Adsorption of phosphates by colloidal alumina*

SALT USED	TIME	METAL ADSORBED BY 1 GM. GEL			PO <sub>4</sub> ADSORBED BY 1 GM. GEL		
		0.025 N	0.05 N	0.1 N	0.025 N	0.05 N	0.1 N
	days	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
KH <sub>2</sub> PO <sub>4</sub> .....	1	5.5	22.4	46.8	137.7	194.7	284.5
	3	14.0	36.4	69.7	186.4	250.5	356.1
	4	14.0	38.8	74.3	186.4	250.5	356.1
MgH <sub>2</sub> (PO <sub>4</sub> ) <sub>2</sub> .....	1	12.1	15.5	19.3	164.4	264.5	316.3
	7	15.4	21.0	26.5	251.9	333.7	414.6
	14	17.2	24.1	29.8	260.5	356.7	444.8
	21	17.2	24.4	30.0	260.5	356.7	447.8
CaH <sub>4</sub> (PO <sub>4</sub> ) <sub>2</sub> .....	1	8.8	17.2	23.2	169.7	236.3	305.0
	3	32.7	51.4	84.9	272.6	393.4	610.2
	4	32.7	51.4	84.9	272.6	393.4	610.2

more shaking to establish equilibrium. These facts are more clearly brought out in tables 2 and 3, and by figures 1 and 2.

By using a continuous mechanical shaking process in the case of calcium and

potassium acid phosphates with aluminum hydrogel, the time required to establish equilibrium was cut down from six weeks to three days. Practically the same final equilibrium point was reached whether shaken by hand or by machine.

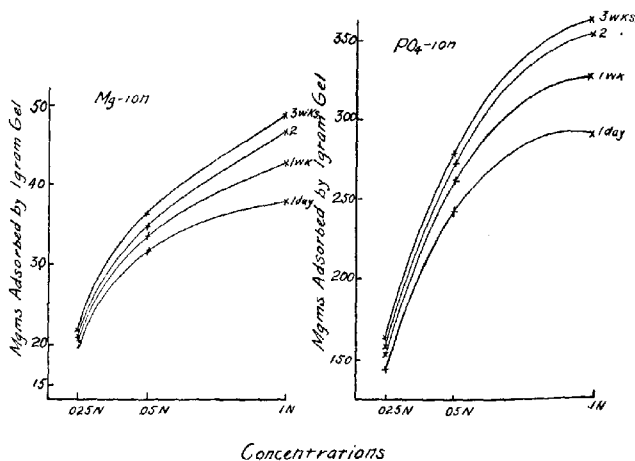


FIG. 1.

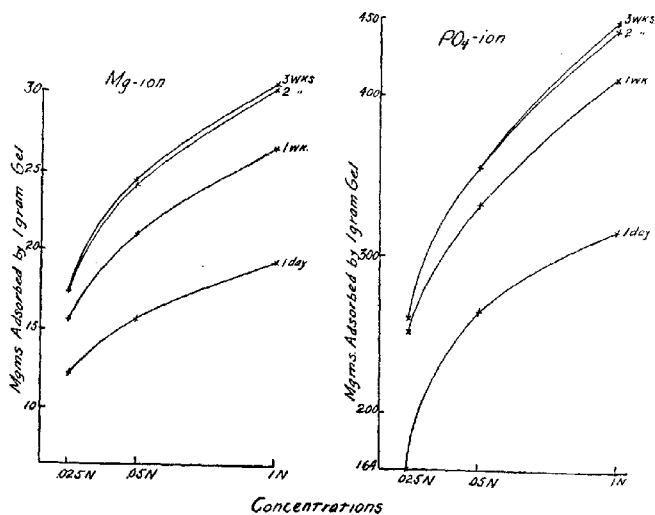


FIG. 2

The curves in figures 1 and 2 show how the adsorption varies with an increase of concentration. It can be observed by the tables that the other salts would give curves similar to the magnesium acid phosphate curves.

The slow establishment of the true equilibrium might be explained by the fact that the equilibrium occurs in two stages; first, the diffusion of the salt in the water contained in the hydrogel, and, second, adsorption itself. It is quite likely that the structure of the gel would prevent a rapid homogeneous distribution of the salt.

The adsorption of phosphates is much more marked by each hydrogel than was the case with the sulfates. In case of the iron hydrogel both ions were adsorbed in approximately equivalent amounts, but the aluminum hydrogel showed a greater adsorption of the phosphate ion than of the metallic ion. The order of the adsorption was the same as in the case of the sulfates.

After finding this marked adsorption of certain plant nutrition salts by the hydrogels, it became of interest to try to release the salts. In view of this the gels which had suffered their maximum adsorption from a .05 *N* solution of calcium acid phosphate were washed successively with 50-cc. portions of distilled water. After each 500 cc. of water had passed through the gel, a 50-cc. portion of the filtrate was taken for analysis. The absorbed nitrates were quickly washed out, the sulfates less quickly, while the phosphates were held persistently, as shown in tables 4 and 5.

The gels thus washed were further leached with certain solutions of acids, bases and salts. The salts used were .05 *N* solutions of calcium, magnesium and potassium sulfates. Analysis of the filtrates showed that no adsorbed material was released by these washings. This was expected since calcium acid phosphate is made up of the two ions which had suffered the greatest adsorption, and hence should not have been released by the less strongly adsorbed sulfate salts.

The acids and bases gave more varied results; .01 *N* solutions of sodium hydroxide, ammonium hydroxide, calcium hydroxide, citric acid, and a saturated solution of  $\text{CO}_2$  were used for washing the gel and successive 50-cc. portions of the filtrate were analyzed. The results are given in table 6.

In case of aluminum all the washings show an increase which rises rapidly in some cases and then decreases. In the washings with sodium hydroxide, ammonium hydroxide and citric acid, the gel was peptized, passing through into the filtrate, and this undoubtedly accounted for at least part of the amount of phosphate in the filtrate. The calcium hydroxide and carbon dioxide solutions gave no peptization, and the increased phosphate in the filtrate was much less marked than in the case of the sodium hydroxide, ammonium hydroxide and citric acid.

There was only slight peptization in the case of iron gel and no marked increase of phosphate in 50-cc. portions of the filtrate. Sodium hydroxide and ammonium hydroxide both slightly increased the phosphate content of the filtrate while there was no increase at all with calcium hydroxide.



It had been found, as stated above, that sulfates failed to release any phosphate and it was further stated why this was anticipated. Conversely, it was suspected that the phosphate would release the sulfates, and hence the replacement of sulfates by phosphates was next tried. Two-hundred cubic centimeter of .05 *N* solutions of potassium sulfate, magnesium sulfate and calcium sulfate were placed in glass stoppered bottles, the equivalents of 1 gm. of dry gel added and the solutions were shaken until equilibrium was established. The adsorption of sulfates was determined, and then sufficient dry magnesium acid phosphate was added to make each solution .05 *N* in regard

TABLE 4  
*Quantity of phosphate found in 50 cc. portions of filtrate*

FILTRATE NUMBER	PO <sub>4</sub> IN 50 CC. OF FILTRATE FROM THE HYDROGELS AT THE END OF 500-CC. WASHINGS	
	Iron	Aluminum
	mgm.	mgm.
1	6.0	10.0
2	3.2	6.4
3	1.5	2.5
4	1.3	2.1
5	1.2	1.6
6	1.0	1.2
7	0.8	1.1
8	0.7	1.0
9	0.7	0.7
10	0.6	0.4

TABLE 5  
*Quantity of phosphate washed from gel*

	PO <sub>4</sub> ADSORBED BY HYDROGEL OF FERRIC OXIDE	PO <sub>4</sub> ADSORBED BY HYDROGEL OF ALUMINA
	mgm.	mgm.
Before washing.....	239.0	162.4
After washing.....	164.0	117.5

to the latter salt. Equilibrium was again established by shaking and the adsorption of each ion in the solution determined. The results are given in table 7.

Table 7 shows that the sulfate ion was entirely replaced by the phosphate ion in both hydrogels. The phosphate ion was less adsorbed by the aluminum gel in the presence of the sulfate radical than when not in the presence of it, except in the case of calcium sulfate, where it was slightly greater. This may have been due to the influence of the calcium ion which is the most strongly adsorbed of the positive ions used. In the case of the iron gel there was an increase of adsorption in the presence of the sulfates over that found when the

same concentration of magnesium acid phosphate was used alone. This increase was greatest in the presence of calcium sulfate and least in the presence of potassium sulfate.

Since the adsorption varied with concentration as shown in table 3 it became of interest to take a 0.1 *N* solution, obtain the maximum adsorption from it by the hydrogels and then dilute the solution to .05 *N* and determine whether this corresponded to the adsorption from a solution which was originally .05 *N*. This was carried out with solutions of magnesium sulfate, magnesium acid phosphate, calcium acid phosphate, and potassium acid phosphate. The results showed that the equilibrium applied only to the portion of the phosphate

TABLE 6  
*PO<sub>4</sub> removed by washing the hydrogels of alumina and ferric oxide with 50 cc. portions of acid and basic solutions*

FIL- TRATE NUMBER	HYDROGEL OF ALUMINA					HYDROGEL OF FERRIC OXIDE				
	NaOH	NH <sub>4</sub> OH	Ca(OH) <sub>2</sub>	Citric acid	Sat. sol. CO <sub>2</sub>	NaOH	NH <sub>4</sub> OH	Ca(OH) <sub>2</sub>	Citric acid	Sat. sol. CO <sub>2</sub>
	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
1	8.1	5.4	1.4	2.5	7.7	4.1	2.5	0.0	0.8	0.6
2	11.3	6.5	2.0	4.1	8.6	10.2	5.5	0.0	0.7	0.6
3	12.2	7.8	1.8	11.6	16.0	12.3	6.6		0.7	0.6
4	13.0	8.4	1.8	14.9	16.2	11.5	5.5		0.7	0.5
5	12.3	9.9	1.7	12.7	17.3	11.2	4.4			
6	12.3	9.4	1.6	12.2	16.0	10.0	4.2			
7		7.6	1.9	10.2	17.6	7.0	4.0			
8		6.0	1.8	7.1		3.5	3.5			
9		6.0					3.0			
10							2.5			

which might be leached from the gel as given in table 5, *i.e.*, the equilibrium applied only to 95.0 mgm. of the PO<sub>4</sub> adsorbed by the iron gel and 44.9 mgm. in case of the aluminum gel.

#### DISCUSSION

In these experiments it has been quite evident that the amount of adsorption depends on how much the coagulated colloid was exposed to the solution. It is probable that the quantity of precipitating electrolyte in the case of colloids has been a varying factor because of the varying speed at which electrolytes are absorbed. The difference in the time required for establishing equilibrium in the case of sulfates and phosphates would bear out this statement.

The nitrates are practically not taken up at all by either hydrogel; hence, it is evident that there can be neither absorption nor chemical reaction between the salt and the hydrogel. In the case of the iron hydrogel we find that the ions are taken up in nearly equivalent quantities, and, hence, it is reasonable to suppose that there must be an adsorption of the molecule as a whole. The

aluminum hydrogel offers a different case, for here we find the phosphate ion taken up in considerable excess of its equivalent of the metal ion with which it is associated.

Much of the data on sulfates and phosphates with both the iron and aluminum hydrogels seems to bear out the theory of selective adsorption. On the other hand when one comes to such results as shown in table 5, one cannot help but believe that there is a chemical force exerting itself. The data shown in table 6 leads one further to believe that this plant food may become useful for plant food by peptization of colloidal gel or by the replacement of one radical by a more strongly adsorbed radical, as shown in table 7.

TABLE 7  
*Replacement of adsorbed  $\text{SO}_4$  by  $\text{PO}_4$  in gels*

	ALUMINA HYDROGEL						FERRIC OXIDE HYDROGEL					
	$\text{K}_2\text{SO}_4$		$\text{MgSO}_4$		$\text{CaSO}_4$		$\text{K}_2\text{SO}_4$		$\text{MgSO}_4$		$\text{CaSO}_4$	
	K	$\text{SO}_4$	Mg	$\text{SO}_4$	Ca	$\text{SO}_4$	K	$\text{SO}_4$	Mg	$\text{SO}_4$	Ca	$\text{SO}_4$
	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
Before adding $\text{MgH}_2(\text{PO}_4)_2$ . . . . .	11.0	19.0	6.2	26.3	5.7	12.0	11.4	13.6	7.6	31.1	12.9	36.0
After adding $\text{MgH}_2(\text{PO}_4)_2$ . . . . .	16.1	None	11.1	None	13.1	None	29.0	None	25.2	None	24.0	None

The work on the equilibrium experiments was interesting from the point of view that the adsorbed material seemed to be held by two distinct forces. About one-third of the adsorbed phosphate seemed to be held as claimed by Freundlich (3) while the remaining two-thirds seemed to be held in some different way. Analysis showed that it was not at least all as iron or aluminum phosphates. Experiments are now being run to find any further evidence as to just how it is held. Also, experiments are about completed, which show that this phosphate which cannot be leached from the gel with distilled water can be used by a plant in its growth. These results will be reported in a later paper.

#### SUMMARY

1. Nitrates suffered only slight adsorption while the sulfates, and particularly the phosphates, suffered large adsorption.
2. The order of adsorption of the cations is calcium, magnesium, potassium, while the order in the case of the anions is phosphate, sulfate and nitrate.
3. The time required for equilibrium to be established was a function of the amount of shaking and the rate of adsorption.
4. The adsorption was specific.
5. With solutions of the same salt, the amount of adsorption increased with an increase of concentration.

6. The adsorption of a metallic ion depended upon its accompanying negative ion. The converse is true but to a much less degree.

7. The nitrates and sulfates adsorbed by the hydrogels can be entirely leached out by water while only about one-third of the adsorbed phosphates can be removed by leaching with water.

8. Only the water-leachable material showed the adsorption equilibrium law.

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## AVAILABILITY OF POTASSIUM IN ORTHOCLASE FOR PLANT NUTRITION<sup>1,2</sup>

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### INTRODUCTION

Chemical analyses show that potassium is always present in soils and, with the exception of peats, mucks, and light soils, in relatively large quantities.

Experiments conducted at the Pennsylvania Experiment Station by Frear and Erb (24) indicate that, in some soils at least, potassium is not only present in considerable quantities but is available to such a degree as to render fertilization with this element unnecessary. In other soils, however, according to DeTurk (21) the potassium normally present is held in such a form that very little is readily available for the use of farm crops. Such soils respond readily to the use of soluble potassium fertilizers, and as a matter of fact, such applications seem to be necessary for maximum crop production.

While the greater percentage of the soil potassium is held in the form of relatively insoluble silicates such as biotite, muscovite, orthoclase, microcline, etc. and their decomposition products, a part is derived from plant residues. If it were not for the latter source, plants requiring large quantities of potassium probably could not reach full maturity owing to the limited amount of the element going into solution from the native minerals. An experiment by Hopkins and Aumer (27) is of interest in this connection. They found that soil freed of acid-soluble potassium would not, at first, support the growth of plants. As all other nutrient materials were supplied to this particular soil, the plants utilized the potassium of the seed, but death occurred soon afterward because the plants were unable to obtain further supplies of this element from the soil. It was found, however, that if these plants were not removed but incorporated with the soil and the process repeated several times, normal growth could be obtained eventually.

The above experiment does not signify, however, that plants are not capable of utilizing potassium directly from the minerals themselves. Lichens readily obtain a foothold on the most resistant potassium-bearing rocks, such as granites. Rocks of this nature compare, in a measure, to the soil freed of available potassium as used in the experiment already mentioned.

<sup>1</sup> The author takes great pleasure in thanking Dr. Firman E. Bear for the many helpful suggestions which he so kindly gave him in the pursuance of this work.

<sup>2</sup> A thesis submitted to the faculty of the Graduate School of the Ohio State University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy, and published as Contribution No. 2 from the laboratories in Agricultural Chemistry, Pennsylvania Experiment Station.

## OBJECT OF THESE INVESTIGATIONS

In view of the fact that most soils contain enough potassium to supply the requirements of crop plants for an indefinite number of years, and yet the cost of materials supplying potassium reaches an enormous figure annually, it seems desirable to consider more carefully the possibility of increasing the availability of the potassium normally present in the soil. Since the problem is one which bears directly upon plant nutrition it was thought that more satisfactory results could be obtained by the growth and subsequent analysis of a plant possessing a high potassium requirement in an artificial soil of known composition, the potassium of which would be supplied in the form of one of the common minerals found in soils. By insuring the presence of other essential elements in available forms and in quantities sufficient to render potassium the limiting factor in the nutrition of the plant, such an investigation should give, in a measure, the availability of the potassium from the mineral supplying it, providing, of course, no serious detrimental factors should arise. In addition, it was thought that if certain other organic and inorganic substances would be added in such amounts as not to seriously interfere with the normal growth of the plant, their effect on the availability of the potassium might be noted.

It is clear, however, that such a method of investigation presents its own peculiar difficulties, such as lack of aeration, and the possible accumulation of substances harmful to plant growth due to the lack of drainage of the pots holding the artificial soil. Pots having drainage facilities would suffer a loss of soluble potassium not accounted for by plant analysis, and the same would hold true if the other plant nutrients were replaced from time to time. On the other hand, such an experiment should give comparative results, providing all factors influencing plant growth were controlled, to a greater or less degree, as was attempted in this investigation.

## RESULTS OF PREVIOUS INVESTIGATIONS

It has been known for a long time that the ordinary potassium bearing silicates are measurably soluble in water.

This fact has been established as a result of experiments conducted by various investigators among whom may be mentioned the Roger brothers (38), Clarke (13, p. 156), Cushman (15), and Kahlenburg and Lincoln (29). In this connection Cameron and Bell (11, p. 17) declare that water brings about their hydrolysis as evidenced by the alkaline reaction obtained on treating an aqueous solution of the mineral with phenolphthalein. Recently Plummer (37) showed that the solvent action of carbonated water is very much greater than that of distilled water.

The liberation of potassium from its minerals has been accomplished in other ways, e.g., by the use of solutions of ordinary salts. Lemberg (30) and Beyer (7) have demonstrated that various salt solutions produced marked solubility effects on the potassium-bearing silicates.

The investigations cited, and similar experiments performed by André (3), Daubré (17), Johnstone (28), and many others have established the fact that under certain conditions at

least, the potassium minerals are soluble in water, more so in carbonated water, and, if treated with solutions of certain inorganic compounds, their potassium may be replaced.

For a number of years, this problem of the liberation of soil potassium has been attacked in various ways. In 1848 Magnus (33) prepared an artificial soil to which he supplied potassium in the form of powdered feldspar only. With such a medium for plant growth, he was able to bring a crop of barley to complete maturity. Similar experiments were conducted by Aitkin (1), Nilson (34), Ballentine (4) and Feilitzen (22), showing that, under certain conditions, at least, part of the potassium of feldspar could be utilized by certain plants during their growth. Hartwell and Pember (26), however, as a result of extensive experimentation on the use of feldspar in this connection, came to the conclusion that little could be expected from this mineral as a source of available potassium for plant nutrition.

In 1860 Dietrich (18) conducted some experiments to determine the solubility of the soil constituents in various salt solutions. He found that potassium was more readily extracted from the soil by carbonated than by distilled water. Later, Dietrich (19) using 0.05 solutions of sodium chloride, calcium chloride, and calcium hydroxide, found that these solutions dissolved more potassium from soils than did pure water. He also found that solutions of sodium nitrate and sodium carbonate had no apparent effect upon the solubility of the soil potassium.

Fraps (23) claims that sodium sulfate, sodium nitrate or other salts have little effect upon the solubility of potassium in soils, but that lime or organic matter liberates this element to some extent. On the other hand, Lyon and Bizzell (32) place little emphasis on the effect of lime in the liberation of potassium from soils while Wheeler (41, p. 75), in summarizing certain experiments carried on at the Rhode Island Station, concluded that a study of the soils of that state did not justify the assumption that lime is to be considered as an extensive liberant of soil potassium.

#### PLAN OF THE EXPERIMENT

##### *Description of the materials used*

Pure white sand thoroughly leached with tap water and rinsed with distilled water was used throughout these experiments as a medium for plant growth. On analysis it was found to contain but a trace of potassium.

Orthoclase was chosen as the mineral to be used. In order to determine its maximum solubility in water under uniform physical conditions, it was pulverized to such a degree that it would readily pass through a 200-mesh sieve. Twenty-five-gram portions of the powder were transferred to Erlenmeyer flasks of 500 cc. capacity, each containing 250 cc. of distilled water and agitated on a shaking machine. It was found that the solutions contained 28.28 parts per million of potassium when equilibrium was obtained.

Buckwheat was chosen as the plant to be grown for two reasons, first, it is primarily a plant of high potassium requirement and, second, it grows reasonably well under greenhouse conditions.

The potassium content of high grade seeds similar to those planted was found equivalent to 1.16 per cent of their total weight, as determined by the method of Ames and Boltz (2, p. 206).

Earthenware jars were used in these investigations, a half-gallon and a gallon size, to each of which the same weight of sand was added.

The organic and inorganic substances used were chemically pure materials and were found to be practically free of potassium, with the exception of calcium carbonate which contained 1 mgm. of potassium in 25 gm. of the material. Dextrose and starch were chosen in preference to other organic additions mainly because of the fact that they are comparatively free of inorganic material.

A complete nutrient solution, in which the constituent compounds were dissolved and made up to volume separately, was devised. This was found to be acid to phenolphthalein



but alkaline to methyl orange. The weights of the different materials estimated on the water-free basis, the volume of distilled water added, and the amounts of each of the resulting solutions taken for one application are given in table 1.

Distilled water was added to the sand in the jars. Each jar was weighed daily and the water was maintained at a content approximating 14 per cent of the weight of sand. When the nutrient solution was added directly to the jars in the quantities indicated above, the resulting concentration at the optimum water-content of the sand was 36.3 p.p.m. of nitrogen, 51.5 of calcium, 32.7 of phosphorus, 41.7 of potassium, 31.6 of magnesium and a trace of iron.

Solutions of the sodium salts were made, having a volume of such concentration that 10 cc. corresponded to 41.7 p.p.m. of sodium when added to the jars. In like manner solutions of dextrose were made up, just before using, and in such concentration that 10 cc. were equivalent to 100 p.p.m. of this compound.

TABLE 1  
*Composition of nutrient solution*

MATERIAL	AMOUNT	VOLUME	APPLICATION
	gm.	cc.	cc.
(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub> .....	14.4908	8000	10
CaH <sub>4</sub> (PO <sub>4</sub> ) <sub>2</sub> .....	25.9389	8000	10
Ca(NO <sub>3</sub> ) <sub>2</sub> .....	68.5225	8000	10
MgSO <sub>4</sub> .....	99.7879	8000	10
K <sub>2</sub> SO <sub>4</sub> .....	9.1661	2500	10
FeCl <sub>3</sub> .....	0.1000	250	1

#### EXPERIMENTAL

A preliminary experiment was undertaken in order to ascertain the practicability of this kind of investigational work. At the conclusion of this test, a more extended experiment was undertaken along the same lines. On May 26, 1919, the appropriate amounts of sand, alone and with the additions of the insoluble compounds, were weighed out and transferred to each of the 90 jars making nine different series of five duplicate tests each, as shown in table 2.

Fifteen grams of buckwheat were planted after the sand had been brought to its optimum water content. After the plants were well above the sand six uniform plants were selected for growth and the others removed with the exceptions of jars 18 and 20 in which seven plants were accidentally allowed to remain until they had reached such a state of growth that it was considered inadvisable to remove them.

The first application of the nutrients was made on June 2 followed by successive additions on June 9, 16, 23 and 30, and on July 7 and 13. The jars were weighed daily and brought to the optimum water-content, care being taken to prevent undue evaporation by white-washing the interior of the greenhouse roof and by keeping the floors sprinkled morning and afternoon.

On July 14 every plant was severed close to the sand, measured and then placed in a weighed tared envelope with the others of the same jar. After drying, the plants were analyzed, duplicate determinations being made instead of taking a composite sample of each individual treatment.

The data of this experiment are given in tables 3-6.

## DISCUSSION OF RESULTS

A factor that affects the growth of plants either advantageously or disadvantageously is that of the reaction of the nutrient medium. In this connection attention is called to the fact that the complete nutrient solution used in this experiment was quite acid in reaction. In addition, it has been stated by Breazeale and LeClerc (9) that plants growing in the presence of compounds such as were used in this solution, render the solution more acid than originally through selective absorption.

While the effect of a nutrient solution moderately acid in reaction is that of hindering the elongations of root systems, this fact in no wise altered the

TABLE 2  
Additions to the quartz sand medium in which plants were grown  
Each pot contained 2750 gm. sand and other materials indicated

GENERAL TREATMENT	SUPPLEMENTAL TREATMENTS								
	Check	CaCO <sub>3</sub> (25 gm.)	CaSO <sub>4</sub> (25 gm.)	NaCl*	Na <sub>2</sub> SO <sub>4</sub> *	Dextrose† (0.2716 gm.)	Dextrose (0.2716 gm.) + CaCO <sub>3</sub> (25 gm.)	Starch (0.2425 gm.)	Starch (0.2425 gm.) + CaCO <sub>3</sub> (25 gm.)
	jar nos.	jar nos.	jar nos.	jar nos.	jar nos.	jar nos.	jar nos.	jar nos.	jar nos.
Complete nutrients.....	1-2	11-12	21-22	31-32	41-42	51-52	61-62	71-72	81-82
Complete less K.....	3-4	13-14	23-24	33-34	43-44	53-54	63-64	73-74	83-84
Same as 2 plus 25 gm. of ortho-clase.....	5-6	15-16	25-26	35-36	45-46	55-56	65-66	75-76	85-86
Same as 2 plus 50 gm. of ortho-clase.....	7-8	17-18	27-28	37-38	47-48	57-58	67-68	77-78	87-88
Same as 2 plus 75 gm. of ortho-clase.....	9-10	19-20	29-30	39-40	49-50	59-60	69-70	79-80	89-90

\* Equivalent to a total of 0.1123 grams of sodium, one-seventh of this amount constituting one application.

† 0.2716 grams, one-seventh of this amount constituting one application.

ability of the plants of jars 1 and 2, which received the complete nutrients, to absorb the potassium supplied them in available form. These plants contained in their tops an average of 92 mgm. of the 112.3 mgm. of potassium supplied. From the data presented in tables 3, 4, 5 and 6 it seems that orthoclase must have exerted a beneficial effect on the nutrient solution as evidenced by yields and amounts of potassium absorbed by the plants of series 1. A greater amount of dry matter was obtained where 50 and 75 gm. of orthoclase were present than where the complete nutrients were supplied, notwithstanding the fact that less potassium was absorbed by the former plants.

TABLE 3  
Total yield of dry material

JAR NOS.	GENERAL TREATMENT	SERIES 1—CHICK	SERIES 2—CaCO <sub>3</sub>	SERIES 3—CaSO <sub>4</sub>	SERIES 4—NaCl	SERIES 5—Na <sub>2</sub> SO <sub>4</sub>	SERIES 6—DEXTROSE	SERIES 7—DEXTROSE + CaCO <sub>3</sub>	SERIES 8—STARCH	SERIES 9—STARCH + CaCO <sub>3</sub>
Ending in		gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
1	Complete nutrients	4.20	4.31	4.08	3.59	4.21	3.86	5.24	3.76	2.55
2		4.33	4.50	5.05	3.89	3.14	4.22	4.39	3.86	3.51
3	Complete less K	0.99	1.31	1.52	1.14	0.57	1.06	1.81	0.58	1.35
4		1.13	1.40	1.37	1.02	1.07	0.90	2.18	0.47	0.63
5	Same plus 25 gm. or-thoclase	3.23	4.36	3.84	4.25	3.68	2.29	2.98	1.92	3.27
6		3.58	3.83	4.00	4.23	3.24	2.61	2.17	2.36	2.81
7	Same plus 50 gm. or-thoclase	4.36	4.24	5.05	4.65	4.19	3.81	3.38	3.19	4.02
8		4.77	4.88*	5.38	4.82	3.93	3.70	3.60	3.38	4.23
9	Same plus 75 gm. or-thoclase	5.24	5.35	5.03	4.88	3.88	4.20	4.10	3.82	3.88
0		5.11	4.87*	4.44	4.30	3.81	4.06	4.08	3.24	3.93

\* Seven plants.

TABLE 4  
Total potassium absorbed

JAR NOS.	GENERAL TREATMENT	SERIES 1—CHICK	SERIES 2—CaCO <sub>3</sub>	SERIES 3—CaSO <sub>4</sub>	SERIES 4—NaCl	SERIES 5—Na <sub>2</sub> SO <sub>4</sub>	SERIES 6—DEXTROSE	SERIES 7—DEXTROSE + CaCO <sub>3</sub>	SERIES 8—STARCH	SERIES 9—STARCH + CaCO <sub>3</sub>
Ending in		mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
1	Complete nutrients	89	92	97	91	97	94	106	100	90
2		95	94	111	102	98	102	111	96	106
3	Complete less K	7	8	10	10	8	11	14	8	14
4		10	9	10	8	9	9	18	7	8
5	Same plus 25 gm. or-thoclase	24	39	36	36	27	17	23	19	25
6		32	38	35	33	17	22	19	19	22
7	Same plus 50 gm. or-thoclase	54	52	65	57	36	32	31	40	44
8		57	61*	69	51	36	31	29	33	37
9	Same plus 75 gm. or-thoclase	79	89	78	61	40	43	37	45	52
0		78	91*	74	55	35	43	37	45	43

\* Seven plants.

As stated before very little potassium was present in the supplemental materials used, yet the plants of jars 3 and 4 were found to contain an average of 8.5 mgm. as shown in table 4. This absorbed potassium undoubtedly came

TABLE 5

*Average relative weights of dry material\**

GENERAL TREATMENT	SERIES 1—CHECK	SERIES 2—CaCO <sub>3</sub>	SERIES 3—CaSO <sub>4</sub>	SERIES 4—NaCl	SERIES 5—Na <sub>2</sub> SO <sub>4</sub>	SERIES 6—DEXTROSE	SERIES 7—DEXTROSE + CaCO <sub>3</sub>	SERIES 8—STARCH	SERIES 9—STARCH + CaCO <sub>3</sub>
Complete nutrients.....	100	103	107	87	86	95	112	89	71
Complete less K.....	25	31	34	25	19	22	46	12	23
Same as 2 plus 25 gm. of orthoclase.....	80	96	91	100	81	57	60	50	71
Same as 2 plus 50 gm. of orthoclase.....	107	107†	122	111	95	88	81	77	96
Same as 2 plus 75 gm. of orthoclase.....	121	120†	111	108	90	97	96	82	91

\* The yield of plants receiving complete nutrients was taken as 100.

† Thirteen plants.

TABLE 6

*Average relative amounts of potassium absorbed\**

GENERAL TREATMENT	SERIES 1—CHECK	SERIES 2—CaCO <sub>3</sub>	SERIES 3—CaSO <sub>4</sub>	SERIES 4—NaCl	SERIES 5—Na <sub>2</sub> SO <sub>4</sub>	SERIES 6—DEXTROSE	SERIES 7—DEXTROSE + CaCO <sub>3</sub>	SERIES 8—STARCH	SERIES 9—STARCH + CaCO <sub>3</sub>
Complete nutrients.....	100	100	113	105	106	106	118	106	106
Complete less K.....	9	9	10	9	9	10	17	8	12
Same as 2 plus 25 gm. of orthoclase.....	30	42	38	37	16	21	22	20	25
Same as 2 plus 50 gm. of orthoclase.....	60	61†	72	58	39	34	32	39	44
Same as 2 plus 75 gm. of orthoclase.....	85	98†	82	63	40	46	40	49	51

\* Plants receiving complete nutrients were taken as 100.

† Thirteen plants.

to a great extent from the jars themselves. Not more than 2.5 mgm. was present in the seeds. That this amount represents closely the quantity set free in the other jars as well is indicated by the amount of potassium absorbed by the plants of series 1 grown in the presence of varying amounts of orthoclase. Taking the blanks into consideration it would appear that the tendency of the plants was to absorb a quantity of potassium directly proportional to the amount of mineral added.

## EFFECT OF CALCIUM SALTS

The effect of calcium carbonate on the absorption of potassium from orthoclase is shown by the plants of series 2, tables 3 and 4. The average amount of potassium taken up by the plants receiving the complete nutrients in addition to calcium carbonate, i.e., those of jars 11 and 12 was approximately the same as in the absence of the lime salt.

The check plants of this series receiving the potassium-free nutrient solution in addition to calcium carbonate, as compared with those of series 1, showed that no more potassium was absorbed than in the absence of the latter addition. On subtraction of this blank, however, it is evident that more potassium was taken up by the plants from orthoclase in the presence of carbonate of lime. The plants of jars 15 and 16 absorbed an average of 30 mgm. of potassium from the mineral or 10.5 mgm. more than the plants of jars 5 and 6. Where increasing amounts of orthoclase were used in addition to the calcium carbonate treatment, the increased absorption of potassium was almost in direct proportion to the amount of mineral added, and in all probability would have been more nearly so if the extra root system of the additional plants in jars 18 and 20 were taken into consideration.

The reason for the better growth of the plants of this series may be partly due to calcium functioning as a plant nutrient but in all probability most of its effect can be attributed to its neutralizing action on the acidity of the nutrient medium, thereby rendering a more favorable condition for plant growth. In series 1 and 2 it is noted that the increasing growth obtained where orthoclase was present was also accompanied by an increase in the amount of potassium absorbed. In both series, however, greater growth was obtained where orthoclase was present notwithstanding the fact that more potassium was taken up from the complete nutrients. In all probability, the increased absorption of potassium was due primarily to the greater growth of the plants, inasmuch as plants grown under favorable conditions should have a greater feeding capacity than plants grown under conditions less favorable.

That plants grown under favorable conditions would absorb more potassium from orthoclase than those grown in an unsatisfactory medium is evident from results obtained by investigations conducted by Cushman and Hubbard (16), Van Bemmelen (6), DeTurk (21), Beaumont (5, p. 17), and others. Their experiments indicate that very little of the potassium obtained on the hydrolysis of a feldspar goes directly into solution but rather it is held in the colloidal covering of hydrated aluminum silicate surrounding each particle in the form of an absorption compound. Assuming then, that each particle of orthoclase becomes covered with colloidal matter, such a film, according to Beaumont (5), will bring into solution a certain amount of potassium, and under certain conditions this is a fairly constant quantity. If, however, plant roots come into contact with such a film, part of the potassium will be removed and the equilibrium destroyed. The tendency, therefore, would be for more potas-

sium to be made available from the mineral and to replace the amount removed by the plant. If this is true, it would appear that the amount of potassium absorbed from orthoclase depends upon the extent of root development and the greater the development the more of the element absorbed. Such a condition naturally arises where the medium is more favorable for plant growth.

There is a marked distinction between the relative availability of the potassium when supplied as the sulfate or mineral. A study of the plants of the different series as shown in table 4 reveals the fact that all plants receiving the complete nutrients with or without other supplementary treatments absorbed practically all of the potassium, and that this element was taken up regardless of the general character of the plants themselves. This fact indicates that water-soluble potassium can be taken quantitatively from a sandy medium and that the amount absorbed is not dependent upon the relative extent of root development. A further study of these particular plants shows, that those of inferior growth contained relatively more potassium in their tops than those more normal in character. In all probability, where the conditions were less suitable for maximum development those plants had a less extensive root growth and consequently less potassium would be found in the roots and more in the tops. These facts show that if potassium goes directly into solution it may be taken up by plants regardless of their general character and this would hold true for the potassium of orthoclase as well as the sulfate. If no interfering factors were present all the plants grown in the presence of varying amounts of orthoclase would absorb relative quantities of potassium and, if the solubility of this element was affected by certain additions, then the amount of potassium absorbed would indicate the relative efficiency of the various additions in this connection. As such results were not obtained it is clear that certain other factors interfered and in all likelihood one was the colloidal matter of the partly hydrolyzed orthoclase.

The plants of jars 21 and 22 receiving the complete nutrients plus 25 gm. of calcium sulfate are shown to have more potassium in their tops than those receiving the complete nutrients alone or with calcium carbonate. This is probably due to a less developed root system and the excess potassium, instead of being utilized for such growth in the connection, is taken up by the main portion of the plants. Plants absorb more of the essential elements than they ordinarily require and at the same time they manifest a selective absorption in regard to certain radicals. The tendency of a nutrient solution containing calcium sulfate when used for plant growth is to become more acid in reaction due to the fact that calcium is more readily absorbed than the sulfate radical. Pitz (36) has found that small amounts of calcium sulfate greatly increased the extent of root systems but for some reason larger concentration had the opposite effect. While the solubility of calcium sulfate is quite low in distilled water, potassium sulfate, according to Cameron and Bell (12, p. 9) increases its solubility to a great extent. This would result in

more calcium being absorbed and the acidity of the nutrient medium to increase with the possibility of such an increase in acidity being great enough to interfere with normal plant growth.

The plants of this particular series show that those of jars 25 and 26 absorbed more potassium from 25 gm. of orthoclase than was absorbed from the same amount of the mineral through any other treatment. Those of jars 27 and 28 not only absorbed more potassium from 50 gm. of orthoclase than with any other treatment, but gave the highest yield of dry matter of the entire experiment as shown in tables 5 and 6. The nutrient medium in this particular instance must have been of optimum reaction and concentration.

Those plants of jars 29 and 30 grown in the presence of 75 gm. of orthoclase, however, gave a lower yield of dry matter than the plants just mentioned and relatively less potassium was absorbed, undoubtedly because the feeding powers of the plant were impaired to the same extent and by the same causes as stated for the case of the plants of jars 21 and 22; or the action may have been due to an accumulation of dissolved calcium sulfate.

It is evident that under certain conditions calcium sulfate aids in the absorption of potassium from orthoclase but that increased absorption is probably due to the increased growth of the plants under these conditions rather than direct replacement of potassium by calcium from the mineral. This statement is in keeping with those of Hart and Tottingham (25), and Pitz (36). That calcium sulfate under other conditions is somewhat injurious to plants has been proved by Fraps (23), and that calcium sulfate in solution does not liberate potassium directly is indicated by the results obtained in experiments conducted by Briggs and Breazeale (10).

#### EFFECT OF SODIUM SALTS

As shown in table 6 those plants of series 4 receiving sodium chloride plus the complete and potassium-free nutrients contained the same amount of potassium as was taken up by those plants grown in the absence of this salt. Where 25 gm. of orthoclase were present, however, better growth was obtained and more potassium was absorbed than in the similarly treated plants of series 1 where sodium chloride was absent. This indicates in a measure at least, that where potassium is present in limited quantities this salt may serve as a substitute, increasing the growth of the plants and indirectly causing an increased absorption of potassium from the mineral.

The plants growing in the presence of 50 gm. of orthoclase and sodium chloride were approximately the same, both in yield and the amount of potassium absorbed, as those grown in the absence of this salt.

This was not true, however, of the plants of this series grown in the presence of 75 gm. of orthoclase, as both the yield and the amount of potassium absorbed were decreased by the sodium chloride.

The effect of sodium sulfate was found to be less satisfactory in comparison with sodium chloride. It must be remembered, however, that relatively

greater amounts of this salt was used than the chloride and it is fairly well known, according to different experiments conducted by Hart and Nottingham (25), Fraps (23) and Breazeale (8) that this salt sometimes has an injurious effect on plants. In some instances this harmful action has been ascribed to the concentrations of the medium rather than to an increase in the hydrogen-ion concentration which might arise through selective absorption by the plants themselves.

#### EFFECT OF ORGANIC MATTER WITH OR WITHOUT CALCIUM CARBONATE

Application of dextrose seriously interfered with the growth and potassium absorption of the plants of series 6 as shown by tables 5 and 6. That such an effect is not altogether unusual has been proved by Lipman and others (31). They found that dextrose applied in a somewhat similar manner depressed the yield of plants.

The too rapid evolution of carbon dioxide, one of the products formed in the decomposition of dextrose, tends to drive out the oxygen from the nutrient solution. According to Snow (39), lack of oxygen seriously interferes with root-hair development of plants, thereby decreasing its absorption powers to some extent. Noyes and Weghorst (35) are of the opinion that carbon dioxide is detrimental to root growth and in fact De Saussure (20) as early as 1804, found that plants grown in pure carbonated water were injured to a considerable extent.

Other acids are also found as decomposition products of dextrose and it was thought that applications of calcium carbonate would tend to neutralize them and so prevent an unsuitable reaction of the nutrient medium. As a matter of fact, such additions failed to exert a pronounced beneficial effect on either the growth of the plants or the amount of potassium absorbed as shown by the data of tables 5 and 6, series 7.

The reasons for these results may be an accumulation of soluble calcium compounds formed by the reaction of calcium with the products of organic decomposition or, perhaps, as the calcium was made available sufficient quantities were absorbed by the plants to seriously interfere with their normal activities. In the latter connection Clevenger (14) found that the hydrogen-ion concentration of the sap of buckwheat plants was lessened in every instance where lime was supplied to the nutrient medium. It is perfectly obvious that a lowering of the concentration of hydrogen-ion would result in affecting the general well being of plants.

Truog (40) found that additions of lime to acid soils sometimes retarded the growth of crops immediately following, such as oats, wheat and corn, which have low lime requirements. He says, however, that this is due to temporary conditions which soon disappear and the lime then greatly benefits these crops as well as those with high lime requirements.

The data concerning the plants of series 8 as shown in tables 4 and 5 indicate that the deleterious effect of starch when used alone on plant growth is more



pronounced than those of dextrose. It is shown, however, where calcium carbonate was used with starch that in a measure at least, the yields and the amounts of potassium absorbed were increased.

From the result of the experiment it is impossible to say whether or not organic matter liberates potassium from orthoclase, as the use of organic substances under the conditions of this experiment shows that such substances seriously interfere with plant growth and thereby obscure their effect on the mineral itself.

#### SUMMARY AND CONCLUSIONS

This investigation had for its purpose the testing of the rate of availability of the potassium of orthoclase when supplied in finely divided form to buckwheat grown in quartz sand cultures.

Twenty-five-gram portions of the orthoclase, pulverized to pass through a 200-mesh sieve, when subjected to the solvent action of distilled water were found to yield soluble potassium at the rate of 282.2 parts per million at the point of equilibrium between solute and solvent. This was equivalent to 7 mgm. of potassium.

This orthoclase was added in 25-, 50- and 75-gram portions and intimately mixed with 2750 gm. of sand to which the other necessary plant nutrients had been added in available forms.

Availability of the potassium of the orthoclase was estimated from the dry weights of the buckwheat plants and from the amounts of the element absorbed by them when the orthoclase was the only source of potassium as compared to the results secured from the use of water-soluble potassium.

The effects of supplemental additions of calcium carbonate and sulfate, of sodium chloride and sulfate and of glucose and starch on the rate of availability of the potassium of the orthoclase measured as indicated above were also studied.

As a result of these studies the following conclusions seem justified:

1. Fifty-gram portions of orthoclase supplied potassium at a sufficiently rapid rate to satisfy the requirements of 7 per cent larger yields of dry matter than were produced by the complete nutrient solution. With 75 gm. of orthoclase the dry weights of plants were 21 per cent greater than those produced by the use of the complete nutrient solution.
2. The plants grown in pots in which the potassium was supplied as orthoclase contained only 30, 60 and 85 per cent as much potassium for the 25-, 50- and 75-gram portions of orthoclase, respectively, as were contained in the plants grown in the complete nutrient solution.
3. The total amount of potassium available from the orthoclase was in no case as large as that in the nutrient solution but the physiological balance of the solution in the sand culture was apparently favorable to a more economical use of the element.

4. Calcium carbonate and sulfate tended to increase the quantity of available potassium. They also aided in the production of a larger amount of dry matter as long as the lack of potassium was a limiting factor in the growth of the plants.

5. When the lack of available potassium was a limiting factor sodium chloride tended to increase the weights of dry matter but on the whole reduced the amount of available potassium. The effect of sodium sulfate was negative both as to weight of dry matter and amount of potassium absorbed.

6. Dextrose and starch tended to reduce the weight of dry matter and of potassium absorbed. The addition of calcium carbonate to these materials did not materially affect the results with dextrose but resulted both in an increase in yield and of potassium absorbed with starch.

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# A COMPARISON OF THE ABSORPTION OF INORGANIC ELEMENTS, AND OF THE BUFFER SYSTEMS OF LEGUMES AND NON-LEG- UMES, AND ITS BEARING UPON EXISTING THEORIES<sup>1</sup>

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## INTRODUCTION

The primary object of these experiments was to study the selective action of different plants as measured by the proportions in which they absorb different elements from soils and nutrient solutions. Little has yet been done by way of comparing the absorption by different plant species when grown under carefully controlled environmental conditions. Considerable work directly or indirectly concerned with the absorption of inorganic elements by plants, including determinations of the ash content and of the effects of different nutrients upon growth, has recently been reported (4, 5, 6, 11, 12, 14). The composition of barley grown on two different soils was studied at successive stages of growth by Burd (1) and the absorption by barley from a nutrient solution was measured at successive stages of growth by Hoagland (9). In these cases, however, the investigators were not studying the relative absorption by different plant species.

By growing plants in nutrient solutions, a very important environmental factor may be controlled in a manner which was not possible a few years ago, when less was known about such solutions.

For these experiments, it was thought advisable to take plants which under ordinary field conditions are believed to differ considerably in composition and to grow them under more carefully controlled conditions, in order to find out whether the differences are really characteristic, or simply the result of different environments.

It has been quite generally noted that legumes, as a rule, have a higher nitrogen content than non-legumes. This is usually accounted for by the fact that, in the field, legumes probably obtain a larger supply of nitrogen than non-legumes by reason of their symbiotic relationship with nitrogen-fixing bacteria. It has been observed further, by certain workers (14) that legumes and other plants with a relatively high nitrogen content have also a correspondingly high calcium content. Because these differences in composition

<sup>1</sup> This paper forms a portion of a thesis presented in partial fulfillment of the requirements for the degree of Ph.D., University of California. The work was carried out at the suggestion of Prof. D. R. Hoagland.

had been noted, it was considered especially desirable, in studying the selective action of different plants, to compare the absorption by legumes and non-legumes.

A second object of these experiments was to examine the theory advanced by Parker and Truog, regarding calcium and nitrogen relationships in plants (14). According to this theory, the building up of proteins in plants involves the production of by-product organic acids, which are neutralized and precipitated by calcium. Hence the amount of calcium taken up is somewhat directly proportional to the amount of protein or nitrogen built up. Since this involves a question of the relative amounts of calcium and nitrogen absorbed by plants, it was considered that the theory could very well be examined in conjunction with other absorption studies.

That part of the legume nitrogen supply which is fixed by nodule bacteria must almost certainly pass into the plant roots proper in a form which is different from the form or forms in which non-legumes obtain their whole supply. The first product of nitrogen assimilation by legume bacteria and the form or forms of nitrogen assimilated by the plant from the nodules are unknown. However, the fact that legumes grown in nutrient solutions do not absorb their nitrogen in the form elaborated by nodule organisms should hardly affect the absorption of other elements. Strowd (16) stated that an increase in the supply of nitrogen, either from nitrates or nodules, causes an increase in amine and amide nitrogen in the soybean plant, but that this increase is independent of the form of nitrogen supplied. Hence there is no evidence as yet that nitrogen metabolism in the plant follows an appreciably different course when the form of nitrogen taken in by the plant is different, and it is not likely that the requirements for other elements would be affected by the form of nitrogen absorbed. Moreover, in one experiment with peas, described later in the paper, the source of nitrogen was mainly derived from the root nodules.

Another object of the experiments was to study the plant sap of legumes and non-legumes, with special reference to calcium absorption. In connection with the belief that the function of calcium is to neutralize and precipitate organic acids present in the plant and thus avoid an excess of acid in the plant sap, determinations were made of the actual acidities, buffer values, and composition of juices of legumes and non-legumes grown in solutions with a plentiful supply of calcium, and in solutions with a limited supply.

The general type of experimentation was as follows: In nearly all cases, the legumes and non-legumes were grown side by side in nutrient solutions, soil or sand cultures, and the plants or solutions analyzed at various stages of plant growth. The plants for the sap studies were grown similarly and were frozen immediately after harvesting; later they were thawed out, the expressed juice was titrated with the hydrogen electrode, and samples were analyzed for certain elements.

## DISCUSSION OF METHODS EMPLOYED

In beginning this work, the different species which were to be compared were grown in separate jars of nutrient solution, and absorption was measured by analyzing samples of the solution taken at intervals. Later the plan adopted was to grow the different species together in each jar, and analyze the plants themselves instead of the solution. In this case, of course, it would be useless to analyze the solution. The advantages and disadvantages of each method will now be considered.

One advantage of growing the plants together, rather than separately, is that the different species must then be growing in exactly the same solution at all times, even when the solution itself undergoes change because of the absorption of ions by the plant. Another advantage of growing the plants together and analyzing the plants themselves, rather than the solution, is that the percentage composition of the different species may then be compared. For certain purposes, it seems better to compare percentages than to compare actual amounts absorbed, because the relative rates of growth of the different species may differ at different periods.

All percentage values in the different tables which follow are based upon the dry weight of the plants and not upon their green weights. If percentages had been based upon the green weights of plant tops, the relative values for the different plants would not have been altered much, since in practically all cases the plant tops compared contained similar percentages of moisture. Of course it would be unreasonable to base any percentages upon the wet weights of the roots, since this depends upon the amount of adhering water, as well as upon their moisture content. These considerations are mentioned since it might be argued that the concentration of the nutrient elements in the plant sap would depend upon the plant's water content.

In interpreting the data obtained by measuring absorption with the two different types of cultures, it will not be possible to compare percentages, because in one case percentages could not be calculated. Ratios of ion equivalents absorbed may be compared because it was possible to calculate these ratios no matter how the absorption was measured. But ratios of ion equivalents absorbed are affected, as shown later, by differences in the solution in which the plants are growing, and may, therefore, be affected by changes in the solution caused by absorption. Evidently, then, ratios obtained by measuring the absorption by different species grown separately are not strictly comparable.

A disadvantage of growing different species together in single containers, as compared to growing them separately, is that a separate lot of plants must be grown for each stage at which the absorption is measured. If plants are analyzed at several growth stages, it means that a very large number of plants must be grown, since variability errors may be large if too few plants are taken for analysis at each stage. When grown separately, of course, nutrient solution samples may be taken as often as desired throughout the whole growth cycle, from one lot of plants.

The possibility of toxic excretions from the roots of plants has often been discussed, and the question was raised as to whether in these experiments one species would excrete a substance from its roots which would be toxic to another species growing along with it. The different species grown together appeared to develop quite normally, however, and there was no evidence of a toxic effect of one upon another.

Evidence has been obtained that plants adjust the reaction of the solution in which they are growing, thus attaining some sort of an equilibrium with it, and it may be that optimum equilibrium conditions for one species are not quite optimum conditions for another species. In this case, it might be impossible for either species to attain equilibrium with the solution if different species were grown together.

However, when the main object of the experiment is to study the relative absorption under a given set of environmental conditions, the advantages of growing the different species separately are certainly outweighed by the advantages of growing them together.

For these selective absorption experiments, plants were chosen which did not differ radically in type of growth, because, for purposes of comparison it is probably well to choose different species which develop at somewhat similar rates, and which come to maturity in about the same length of time. The more nearly alike the different species are in rate of growth the better, no doubt, will be the comparison of their relative absorption of ions at any particular stage of growth. Since it is not probable that any two species follow exactly the same growth cycle, at best plants can only be compared at approximately the same stage, or stages, of growth. It was thought, however, that the plant's composition would change but little throughout a considerable period of vegetative growth if the nutrient solution were maintained somewhat constant in composition.

Details of each experiment will be found attached to the different tables. The number of plants grown for analysis at each stage of growth was fairly large in order to reduce errors resulting from variability. The number taken to make up a composite sample was in no case less than twenty, and in most cases much larger. The importance of errors resulting from the variability of individual plants has been emphasized by Davis (4). Care was taken, also, to change the solutions often enough to avoid complete absorption of any one constituent at any time, except in special cases, as, for example, when a solution of low calcium content was used. A few drops of iron solution were added every three or four days. The importance of these precautions has been emphasized by Hoagland (9, 10).

The compositions of the nutrient solutions employed are given below. "Parts per million" refer to the number of grams of each ion per million grams of solution. Under "relative numbers of ion equivalents" each value given represents the percentage of the particular ion equivalent referred to, in relation to the sum of the five or six different ion equivalents considered in the

table. The advantage of this method of representation is that it shows the number of each of the ions or ion equivalents in relation to the total number of ions or ion equivalents present in the solution, as well as relations between particular ions.

TABLE I  
*Composition of different nutrient solutions employed*

	Ca	K	Mg	NO <sub>3</sub>	PO <sub>4</sub>	SO <sub>4</sub>
<i>Parts per million</i>						
* Control solution .....	160	185	55	720	105	215
Low Ca solution .....	18	355	5	486	52	100
Low N solution .....	100	100	30	192	134	242
<i>Relative number of ion equivalents present</i>						
Control solution .....	22	13	12	32	9	12
Low Ca solution .....	4	41	2	36	7	10
Low N solution .....	22	11	11	14	19	23

\* General nutrient solution used by Hoagland and found adapted to the normal growth of many types of plants, in solution or sand culture.

# DISCUSSION OF RESULTS BEARING UPON THE THEORY ADVANCED BY PARKER AND TRUOG

Parker and Truog gathered data from many sources regarding the percentages of calcium, nitrogen, potassium, magnesium and phosphorus in different plants. They then listed the plants in order of increasing percentages of nitrogen, and found that they had, with but few exceptions, arranged them in order of increasing percentages of calcium also, but not in any orderly relationship with the percentages of the other elements. In order to explain this apparent parallel relationship between the amounts of nitrogen and calcium taken up, the theory was advanced that the building up of proteins in plants involves the production of by-product organic acids, which are neutralized and precipitated by calcium, and that practically all of the calcium taken up by plants is utilized in this way.

Before considering experimental results bearing upon this calcium and nitrogen relationship theory, it would probably be well to mention one or two of the possible objections to the theory. In the first place, the soils upon which these plants were grown were unknown to Parker and Truog, and it may have happened that most of the plants containing high percentages of nitrogen were grown on soils rich in calcium. For example, we know that legumes are usually richer in nitrogen than non-legumes, and many farmers consider that legumes must be grown on soils rich in lime. Then there seems to be no particular reason for thinking that protein metabolism would produce more by-product acids than carbohydrate metabolism.



With the object of finding out whether the amount of nitrogen utilized by plants is depressed by a limited supply of calcium, barley and peas are grown together in a nutrient solution containing little calcium or magnesium, but a good supply of the other essential elements, as well as in a balanced nutrient

TABLE 2

*Data on barley and peas grown in the control nutrient solution, and in a solution deficient in calcium*

CROP	NUTRIENT SOLUTION	Ca	K	Mg	N	P
<i>Percentage (dry weight) composition</i>						
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Barley tops.....	Control	1.02	4.93	0.39	4.65	1.04
Pea tops.....	Control	1.19	5.19	0.37	4.46	0.87
Barley tops.....	Low calcium	0.74	8.11	0.38	5.75	1.10
Pea tops.....	Low calcium	0.78	7.58	0.31	5.91	0.99
Barley roots.....	Control	0.99	6.46	0.38	4.37	2.00
Pea roots.....	Control	0.95	6.61	1.13	4.18	2.19
Barley roots.....	Low calcium	0.39	8.15	0.54	4.08	1.28
Pea roots.....	Low calcium	0.39	11.02	0.56	4.57	1.43
Barley plants.....	Control	1.01	5.24	0.39	4.60	1.19
Pea plants.....	Control	1.14	5.47	0.53	4.39	1.14
Barley plants.....	Low calcium	0.67	8.08	0.41	5.43	1.14
Pea plants.....	Low calcium	0.70	8.27	0.36	5.62	1.08
<i>Relative ion equivalents absorbed</i>						
Barley plants.....	Control	8	20	5	50	17
Pea plants.....	Control	9	21	6	47	17
Barley plants.....	Low calcium	5	26	5	50	14
Pea plants.....	Low calcium	5	27	4	51	13

*Details of experiment*

Control solution:

75 barley and 75 pea plants grown 48 days.

Low calcium solution:

75 barley and 75 pea plants grown 33 days.

In both cases, 3 barley and 3 pea plants were grown together in each jar.

solution. Another object of this experiment was to determine whether barley and peas would be affected differently by altering the composition of the nutrient solution. The details of the experiment and the results obtained follow in table 2.

Comparisons of percentage composition (table 2) and comparisons of the relative numbers of ion equivalents absorbed show that barley and peas

resemble each other quite closely in type of absorption. The absorption is changed alike for both by using a different nutrient solution, and evidently the amounts of certain elements absorbed vary somewhat directly as the concentration of those elements in the nutrient solution. Thus barley and peas grown in the low calcium solution contained distinctly less calcium and more potassium, as the solution itself contained less calcium and more potassium. True, the percentages of nitrogen, magnesium and phosphorous apparently did not vary directly as their concentration in the solution, but the amounts of these elements absorbed might be affected by the amounts of other elements absorbed, by the rate of growth, by the frequency with which the solutions were changed, and perhaps by still other factors. The point is that the changed solution affected barley and peas alike.

Most legumes, under ordinary field conditions, contain higher percentages of nitrogen than most non-legumes, and, according to Truog's theory, would therefore require a larger calcium supply for normal growth than non-legumes. But when a nutrient solution was used, the peas absorbed about the same relative amounts of nitrogen and calcium as the barley. When a low calcium solution was used, they both absorbed relatively more nitrogen than before, and distinctly less calcium. This suggests that the amount of calcium absorbed varies with the amount of calcium in solution rather than with the amount of nitrogen absorbed.

Further evidence that the building up of proteins does not necessarily involve the taking up of especially large amounts of calcium, or that the amount of calcium taken up is not proportional to the amount of nitrogen taken up, was obtained from an experiment in which barley and peas were grown together in sand to which a low nitrogen solution was added. The pea plants in two of the 5-gallon crocks used were inoculated, and in two others uninoculated. The details of the experiment and the results are given in table 3.

The inoculated pea plants were larger, and had a distinctly higher nitrogen content than the uninoculated, but not a higher calcium content (table 3). As a matter of fact, the uninoculated plants, with the lower nitrogen content had a slightly higher calcium content than the inoculated, but this difference in calcium is probably insignificant. The difference in nitrogen content, however, is undoubtedly significant. Enough plants were grown to minimize differences resulting from the variability of individual plants; this is emphasized by the close agreement in average weight of plants and percentage composition between the barley check plants grown with the uninoculated peas and those grown with the inoculated peas. Obviously, then, the increased nitrogen absorption by inoculated peas did not involve an increased calcium absorption.

#### A COMPARISON OF THE ABSORPTION BY BARLEY AND PEAS FROM A SOIL AND A NUTRIENT SOLUTION

Having found that barley and peas grown together in different nutrient solutions absorbed, in proportion to their weights, practically the same amounts

of calcium, nitrogen and other elements, the question arose whether the same would hold true if they were grown together in a soil. It seemed reasonable to suppose that in a solution, diffusion would be so rapid that the solution for both plants must be exactly the same, but in soil, diffusion would be much

TABLE 3

*Data on peas, inoculated and uninoculated, and of barley, grown in sand cultures to which a solution deficient in nitrogen was added*

CROP	Ca	K	Mg	N	P
<i>Percentage composition</i>					
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Pea tops, uninoculated.....	1.59	5.67	0.47	3.73	0.89
Pea tops, inoculated.....	1.43	5.07	0.50	4.56	1.10
Barley tops, with uninoculated peas.....	1.22	5.47	0.53	3.03	1.07
Barley tops, with inoculated peas.....	1.31	5.74	0.52	3.10	1.14
<i>Absorption per plant</i>					
	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
Pea tops, uninoculated.....	0.0043	0.0154	0.0013	0.0101	0.0024
Pea tops, inoculated.....	0.0056	0.0200	0.0020	0.0180	0.0044
Barley tops, with uninoculated peas.....	0.0025	0.0112	0.0011	0.0062	0.0022
Barley tops, with inoculated peas.....	0.0024	0.0105	0.0010	0.0057	0.0021
<i>Relative ion equivalents absorbed</i>					
Pea tops, uninoculated.....	13	24	6	43	14
Pea tops, inoculated.....	11	19	6	48	16
Barley tops, with uninoculated peas.....	11	25	8	38	18
Barley tops, with inoculated peas.....	11	25	7	38	19

*Details of experiment*

Used four 5-gallon crocks filled with pure quartz sand, to which a low calcium solution was added. The 27 pea plants in two of the crocks were inoculated, and the 27 in the other two were uninoculated; this was successfully accomplished by soaking pea seed in a fresh culture of pea nodule bacteria, in one case, and by soaking pea seed in very dilute formaldehyde in the other case. As many barley plants as pea plants were grown in each jar; these served as checks. The plants were grown outside in July and August, 1921, and were 50 days old when harvested. Four changes of nutrient solution were used, the solution being changed by means of a suction apparatus. The average weights per plant were as follows:

	GREEN TOPS	DRY TOPS
	<i>gm.</i>	<i>gm.</i>
Barley with uninoculated peas.....	0.86	0.21
Barley with inoculated peas.....	0.74	0.18
Uninoculated peas.....	1.7	0.27
Inoculated peas.....	3.7	0.39

slower, and it was thought that, owing to differences in the distribution of the roots together with possible differences in carbon dioxide output from the roots, the two plants might be absorbing from different solutions. Barley and peas were therefore grown together in an adobe soil, and the analytical data obtained from this experiment are presented, for the sake of comparison, along with data obtained when these plants were grown for a similar length of time in a nutrient solution.

TABLE 4  
*Data on barley and peas grown together in the control nutrient solution, and in an adobe soil*

CROP	CULTURE MEDIUM	Ca	K	Mg	N	P
<i>Percentage composition</i>						
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Barley tops.....	Control solution	1.02	4.93	0.39	4.65	1.04
Pea tops.....	Control solution	1.19	5.19	0.38	4.47	0.87
Barley tops.....	Adobe soil	0.67	5.37	0.66	2.88	0.29
Pea tops.....	Adobe soil	1.57	5.93	1.03	3.69	0.37
<i>Absorption per plant</i>						
		<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
Barley tops.....	Control solution	0.0032	0.0155	0.0012	0.0146	0.0032
Pea tops.....	Control solution	0.0043	0.0186	0.0013	0.0160	0.0031
Barley tops.....	Adobe soil	0.0021	0.0171	0.0021	0.0092	0.0009
Pea tops.....	Adobe soil	0.0056	0.0213	0.0037	0.0133	0.0014
<i>Relative ion equivalents absorbed</i>						
Barley tops.....	Control solution	8	20	5	52	15
Pea tops.....	Control solution	9	21	5	51	14
Barley tops.....	Adobe soil	7	27	10	50	6
Pea tops.....	Adobe soil	13	24	14	44	6

*Details of experiment*

Control solution:

75 barley and 75 pea plants, grown 48 days. 3 pea plants and 3 barley plants grown together in each jar.

Soil Culture:

2 5-gallon jars used, 16 barley and 16 pea plants in each. Grown 45 days.

Comparisons of the amounts of calcium absorbed show that when barley and peas were grown together in a nutrient solution, the peas contained about 17 per cent less calcium than the barley, and that when grown together in an adobe soil for a similar length of time, the peas contained approximately 135 per cent more calcium than the barley. It should be noted, also, that the amount of magnesium absorbed by peas was much larger than the amount absorbed by barley when they were grown together in this soil, but that they

both absorbed about the same amount of magnesium from the nutrient solution. The other differences between barley and peas grown together in the soil were not as large, but it is interesting to observe that all were in favor of the peas.

Our next problem was to try to find an explanation for this difference in absorption by barley and peas when grown together in a soil, as contrasted with their similar absorption when grown together in a nutrient solution.

One suggestion was that peas grown in soil have more extensive root systems than barley, and thus obtain more calcium. In a nutrient solution, free from solid particles, diffusion is so rapid that a difference in extent of root systems would not be expected to have an appreciable effect, but in a soil where diffusion is relatively slow, the extent of the root system might be an important factor in determining the amounts of the various elements absorbed. It might be argued, however, that if the differences in calcium absorption were wholly the result of the extent of root system, a difference approximately as great for each of the other elements should be expected. This did not occur except in the case of magnesium. Nevertheless, the results might still be explained on the basis of extent of root systems. It is known that ions diffuse at different rates through a soil, and it may be that calcium and magnesium ions diffuse relatively slowly, and thus greater opportunity for absorption would occur with a more extensive root system. The faster moving ions, on the other hand, might come in contact with the smaller root system as rapidly as the plant could absorb them.

Another suggestion was that peas may give off from their roots more carbon dioxide than barley, and that the carbonic acid thus formed would increase the solubility of calcium and magnesium to a greater extent than that of other nutrient elements, insoluble calcium and magnesium compounds being changed to soluble bicarbonates.

#### A COMPARISON OF THE RELATIVE AMOUNTS OF CARBON DIOXIDE GIVEN OFF BY BARLEY AND PEA ROOTS

The relative amounts of carbon dioxide given off by barley and pea roots were then measured. It should, perhaps, be stated that various other methods of measuring the carbon dioxide output from roots were tried before devising the following simple method, which proved most satisfactory.

One-quart Mason jars were filled up almost to the corks with pure quartz sand, and nutrient solution was added. Six seedlings were planted in each jar, each plant passing through a separate hole in the rubber cork. Through two other holes in the cork glass tubes were inserted just through the cork from above. One was a straight glass tube through which nutrient solution could be poured, and the other was a large-diameter bent glass tube, to the one end of which was attached a flask of standard barium hydrate whenever the carbon dioxide output was to be determined. At these times the open spaces in the cork around each plant were filled with plastic clay, and the tube through which nutrient solution was added was also closed. Two jars of peas and two jars of barley were arranged in this way.

The plants were between 50 and 60 days old when the determinations were made. After making the last carbon dioxide determination, the sand was washed away from the roots, and the plants were weighed. The weights of twelve plants together were as follows:

	GREEN TOPS	DRY TOPS	WET ROOTS	DRY ROOTS
	gm.	gm.	gm.	gm.
Barley.....	46.5	6.1	36.1	3.5
Peas.....	49.9	6.6	36.1	2.0

The barley and pea plants weighed approximately the same, though the dry pea roots weighed less than the dry barley roots.

The unprecipitated barium hydrate was titrated with 0.02 *N* sulfuric acid, using phenolphthalein as the indicator.

	BARLEY		PEAS	
	cc.	cc.	cc.	cc.
0.02 <i>N</i> Ba (OH) <sub>2</sub> neutralized by CO <sub>2</sub> in 45 hours.....	21.5	21.3	40.0	51.2
0.02 <i>N</i> Ba (OH) <sub>2</sub> neutralized by CO <sub>2</sub> in 90 hours.....	46.0	47.8	96.6	81.0

Here we have evidence that under these experimental conditions, peas gave off from their roots, in proportion to the size of the plants, a great deal more carbon dioxide than barley. The amount of carbon dioxide given off from the roots in a soil probably has a great influence upon the solubility of substances in intimate contact with the roots, and particularly upon the solubility of calcium and magnesium. In a nutrient solution, on the other hand, all nutrients are already in solution, and no such effect could be expected. Thus we have a very probable explanation for the dissimilarity in absorption by barley and peas when grown together in a soil, as contrasted with their similar type of absorption when grown together in a nutrient solution. This, however, does not exclude the possibility that differences in extent of root system are in part responsible for the differences in absorption.

In view of our results obtained with barley and peas grown side by side on a single soil, it seems quite probable that if the plants listed by Parker and Truog were grown in a similar fashion, differences in calcium content such as those shown in their table would still be obtained, but it seems more likely that these results depend upon the relative amounts of carbon dioxide given off by the roots, together with variations in extent of root system, than that they are primarily related to the amounts of nitrogen metabolized by the plants.

It should, perhaps, be mentioned that in Parker and Truog's list of 34 plants, which were arranged in order of increasing content of nitrogen and incidentally of calcium, 13 of the first 14 belong to the grass family, and 10 of the last to the legume family.

In an earlier publication (17), Truog presents evidence to show that plants which have naturally a high calcium content, have a high feeding power for phosphorus in rock phosphate; that is, such plants grow almost normally when their only source of phosphorus is rock phosphate, whereas plants which

have naturally a low calcium content do not grow at all well when their only source of phosphorous is rock phosphate. The explanation suggested was that when carbonic acid reacts with rock phosphate two products are formed, one being  $\text{CaHPO}_4$  and the other being  $\text{Ca}(\text{HCO}_3)_2$ , and that plants with the high feeding power for phosphorous in rock phosphate take up both of these compounds whereas plants with a low feeding power take up only the  $\text{CaHPO}_4$ . In this second case  $\text{Ca}(\text{HCO}_3)_2$  accumulates in the soil solution and, according to the law of mass action, depresses the concentration of  $\text{CaHPO}_4$ , and limits the plant's supply of phosphorous. Now it is undoubtedly true that some ions are taken up by plants more readily than other ions, but it seems improbable that there is a sharp distinction between plants which can take up  $\text{Ca}(\text{HCO}_3)_2$  and plants which cannot. It seems more likely that many plants which have naturally a high calcium content and which have greater power than other plants to take up phosphorous from rock phosphate, owe both properties in large measure to the fact that they excrete more carbon dioxide from their roots than other plants.

It is entirely probable that some plants may remove calcium from solution more rapidly than others, because of certain reactions or precipitations occurring in the plant or because of selective powers of the cell. The experiments with beans, to be described presently, suggest that this may sometimes be true. However, since peas and vetch did not show a similar selective action, it is difficult to conclude that legumes in general, or plants with a high calcium content when grown in a soil necessarily have a metabolism of this type.

Reference should, perhaps, be made here to an explanation suggested by Truog for the beneficial effect of lime upon the growth of certain plants, notably legumes (17). The idea advanced was that only calcium in the form of bicarbonate will neutralize and precipitate organic acids formed in the plant. Plants which produce a large amount of by-product organic acids cannot grow normally, therefore, unless calcium in the form of bicarbonate is present in the soil; such plants are benefited by liming because bicarbonates cannot exist in a very acid soil. In these nutrient solution experiments, however, no bicarbonate was present in the solution as first made up, and the legumes grew normally. Of course this would not altogether prove that the plants can grow satisfactorily without bicarbonates, as bicarbonates would soon replace nitrates absorbed from the solution. Experiments conducted by other workers would, however, suggest that bicarbonates are unnecessary. It has been found possible to grow certain legumes satisfactorily in solutions and some soils in which the hydrogen ion concentration maintained is greater than that at which bicarbonates can exist to any extent.<sup>2</sup>

#### A COMPARISON OF THE ABSORPTION BY BARLEY AND COMMON VETCH FROM A SOIL AND A NUTRIENT SOLUTION

Another experiment similar to the one in which comparison was made of the absorption by barley and peas grown in solution and in soil, was conducted

\* These remarks apply to solutions in which nitrogen is supplied in the form of nitrate.

with barley and common vetch. Vetch was chosen because, in view of the results obtained with peas and barley, it seemed particularly desirable to find out how other legumes would compare with barley when grown in a nutrient solution and when grown in a soil. Because of lack of time only calcium and nitrogen determinations were made. The results, which follow, indicate that vetch and peas resemble each other closely in their absorption from soils and from solutions.

Comparisons of percentages show that when the barley and vetch were grown together in a nutrient solution, they absorbed similar amounts of calcium and nitrogen, but that when they were grown together in a soil, the

TABLE 5  
*Calcium and nitrogen content of barley and vetch grown together in the control nutrient solution and in an adobe soil*

CROP	CULTURE MEDIUM	Ca	N
		<i>per cent</i>	<i>per cent</i>
Barley tops.....	Control solution	1.46	4.93
Vetch tops.....	Control solution	1.53	4.57
Barley tops.....	Adobe soil	0.50	3.45
Vetch tops.....	Adobe soil	0.90	4.28

*Details of experiment*

Control solution:

60 barley plants and 60 vetch plants grown for 46 days. Barley and vetch grown together. 3 plants of each per jar (1 liter).

Soil culture:

Barley and vetch grown together 46 days. Total of 29 plants each.

vetch absorbed very much more calcium and somewhat more nitrogen than the barley. The probable reasons for this result were discussed in connection with the pea and barley experiment.

#### DISCUSSION OF OTHER ABSORPTION EXPERIMENTS

In order to study the selective action of barley and peas, as shown by the proportions in which they absorb different elements from a solution which is exactly the same for both, barley and field peas were grown together in the control nutrient solution, and the plants were analyzed at four different stages of growth. The details and figures follow are given in table 6.

It will be noticed, in comparing the four stages, that the relation of ion equivalents was quite different in the final stage from that in the three earlier stages. The potassium and nitrogen percentages were lower in the final stage than in the other stages.

Comparisons of percentage composition (table 6) and of the ratios in which ion equivalents were absorbed, at each different stage, show that barley and



CROP	AGE	COMPOSITION								CROP	ION EQUIVALENT ABSORBED	CROP	NUMBER OF SOIL-TION CHANGES	NUMBER OF PLANTS	PART OF PLANT	AVERAGE WEIGHT PER PLANT							
		Part of plant	Ca	K	Mg	N	P	per cent	per cent							per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Barley	24	Tops	0.94	5.00	0.44	3.93	0.87	Barley	9	20	8	46	17	1400	Tops	0.27	0.031						
Peas		0.84	2.16	0.37	4.84	0.83	Peas	7	13	6	58	16	620	Peas	1.01	0.095							
Barley	48	Roots	1.15	2.60	0.88	2.61	1.29	Barley	8	20	5	50	17	75	Roots	0.12	0.008						
Peas		0.70	6.46	0.83	5.19	1.32	Peas	9	21	6	47	17	75	Peas	0.64	0.029							
Barley	70	Tops	1.02	4.93	0.39	4.65	1.04	Barley	10	23	5	47	15	20	Tops	2.88	0.313						
Peas		1.19	5.19	0.38	4.47	0.87	Peas	10	19	10	45	16	20	Peas	3.12	0.359							
Barley	140	Roots	0.99	6.46	0.38	4.37	2.00	Barley	19	15	8	34	24	20	Roots	1.04	0.072						
Peas		0.92	6.61	1.13	4.18	2.19	Peas	22	13	9	40	16	4	Roots	1.47	0.096							
Barley		Tops	1.27	5.97	0.44	4.38	0.96	Barley	19	15	8	34	24	20	Tops	24.00	2.14						
Peas		1.00	3.64	0.27	3.10	0.60	Peas	22	13	9	40	16	4	Peas	38.70	4.64							
Barley		Roots	1.16	4.99	0.43	3.60	1.54	Barley	19	15	8	34	24	20	Roots	6.94	0.34						
Peas		0.90	4.78	2.35	3.47	1.79	Peas	22	13	9	40	16	4	Peas	17.50	1.02							
Barley		Tops	1.26	2.18	0.39	1.70	0.91	Barley	19	15	8	34	24	20	Tops	29.25	11.10						
Peas		1.47	1.73	0.33	1.80	0.51	Peas	22	13	9	40	16	4	Peas	25.50	7.80							
Barley		Roots	3.81	0.63	0.21	1.75	0.56	Barley	19	15	8	34	24	20	Roots	18.40	0.86						
Peas		1.06	1.28	0.84	2.69	0.85	Peas	22	13	9	40	16	4	Peas	20.60	0.86							

The 24-day old plants were supported, during growth, on parafined netting stretched over the tops of 5-gallon glazed crocks. All others were grown in 1-quart Mason jars. In the case of the 48-day old plants, 3 barley and 3 pea plants were grown in each jar, and in the cases of the older plants, 1 barley and 1 pea plant were grown in each jar. All plants were grown in the greenhouse, January to May, 1921. The solution was not renewed right up to the time of harvesting the 140-day old plants, as it has been found that plants maintain their vegetative growth for a very long time, and do not mature naturally unless partially starved towards maturity. The 140-day old plants were practically mature, but the seed formed was small, and was not analyzed separately. At 70 days, the barley plants had, on the average, 5 to 6 strong tillers, but no heads, and the peas had an average of 1.6 tillers which averaged 56 inches in length, and had just begun to blossom. At 48 days old, the barley plants were just beginning to tiller. The barley and pea plants appeared to grow quite normally at all times.

peas in general resemble each other rather closely in type of absorption. Of course, there are a number of exceptions to this statement, but in this connection, attention is called to the following points.

It can hardly be said that there is a difference in percentage of any one element which holds consistently in favor of either barley or peas throughout all four stages.

The roots were more irregular in composition than the tops, and the largest differences in percentage composition between barley and peas, at any one stage, are to be found in the roots. But the roots form such a small portion of the plant that the average composition of the whole plant, in most cases, resembles closely the average composition of the tops.

The average weights of the barley and pea plants were more nearly alike in the cases of the 48-day old and 140-day old plants than in the cases of the 24-day old and 70-day old plants (table 6), and it will be observed that the composition of the barley and pea plants resemble each other more closely in the former cases. The 24-day old plants were small enough to be influenced in composition by the composition of the parent seed.

The calcium percentages of barley and pea plants, or tops, resemble each other rather closely at every stage. The greatest difference in percentage occurred in the case of the 70-day old plants at which time the barley tops then had about 26 per cent more calcium than the pea tops. When grown together in an adobe soil, the pea tops contained approximately 135 per cent more calcium than the barley, whereas when grown together in this nutrient solution, at a similar age (48 days) and weight, the peas contained only about 17 per cent more calcium than the barley.

Thus the results obtained in this nutrient solution experiment bring out no evidence of a characteristic difference between barley and peas in type of absorption.

In order to gain further information regarding the relative absorption of different plants, some work was conducted with beans and barley.

It will be observed that the bean and barley plants have about the same content of nitrogen, magnesium (tops), and phosphorous, but that the content of calcium and potassium differed markedly. Beans have a higher percentage of calcium and a lower percentage of potassium than barley. Also, in this experiment, beans have a distinctly higher calcium percentage than either barley or peas at any one of the four growth stages in the experiment previously described.

The ratio of ion equivalents, in the case of beans, is about 1 of calcium to 1 of potassium. This is shown in two separate experiments (tables 7 and 8) in one of which the computations were based upon the composition of the solution in which the beans were grown, and in the other based upon the composition of the plants themselves. The ratio of ion equivalents absorbed by barley was about 1 of calcium to 2 or more of potassium in this experiment, and in all but the last stage of growth of both barley and peas in the previously

described experiment. It would be unreasonable to compare the ratio of ion equivalents in these young bean plants with the ratio of ion equivalents in the mature barley and peas. Late stages of growth of the beans were not studied.

TABLE 7  
*Relative ion equivalents absorbed by beans grown in the control nutrient solutions at 2, 4, and 6 weeks old*

AGE	Ca	K	Mg	NO <sub>3</sub>	PO <sub>4</sub>	SO <sub>4</sub>
<i>weeks</i>						
2	22	18	10	22	10	18
4	17	19	10	35	14	5
6	18	18	9	37	11	7

TABLE 8  
*Data on barley and beans grown together in the control nutrient solution*

	Ca	K	Mg	N	P
<i>Percentage composition</i>					
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Barley tops.....	1.60	7.99	0.52	5.04	0.89
Bean tops.....	2.50	4.78	0.57	4.31	0.92
Barley roots.....	0.81	5.92	0.40	4.51	1.65
Bean roots.....	0.86	6.62	1.09	4.75	1.99
<i>Relative ion equivalents absorbed</i>					
Barley plants.....	9	26	6	46	13
Bean plants.....	16	18	7	44	15

*Details of experiment*

Table 7 refers to "Speckled Cranberry" beans which were grown for a period of 6 weeks in the control nutrient solution. Absorption was measured by analyzing the nutrient solution at intervals of two weeks. Twenty one-quart Mason jars were used and two bean plants were grown in each.

Table 8 refers to barley and "Speckled Cranberry" beans grown for a period of 44 days in the control nutrient solution. Twenty-four one-quart Mason jars were used and 3 barley and 3 bean plants were grown in each. Three changes of nutrient solution were used. The average weights of the plants when harvested were as follows:

	GREEN TOPS	DRY TOPS	WET ROOTS	DRY ROOTS
	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
Barley.....	2.18	0.28	1.04	0.04
Beans.....	11.57	1.27	4.28	0.23

It will be seen in the last case that the bean plants were very much larger than the barley plants when harvested and analyzed, and the comparison is, therefore, not as satisfactory as when plants which grow at more nearly equal rates are used.

The evidence at hand thus indicates that there is a characteristic difference in the types of absorption of barley and beans in the early stages at least. Beans absorb more calcium in proportion to potassium, from a given solution, than either barley or peas. No distinctive difference in composition with regard to nitrogen, however, was noted.

A COMPARISON OF THE ACTUAL ACIDITIES, BUFFER VALUES, AND COMPOSITION OF THE SAP OF LEGUMES AND NON-LEGUMES

In connection with the belief that the function of calcium is to neutralize and to precipitate organic acids present in the plant sap, and thus avoid

TABLE 9  
*Composition of plant sap*

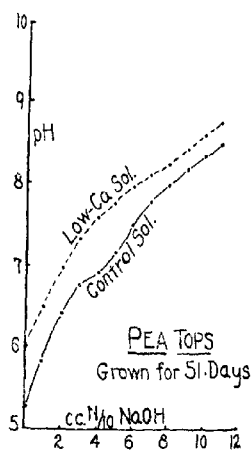
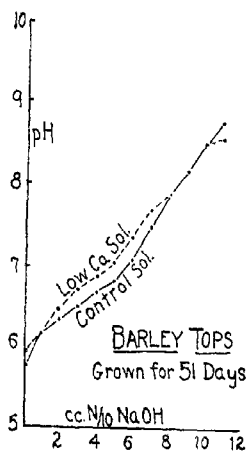
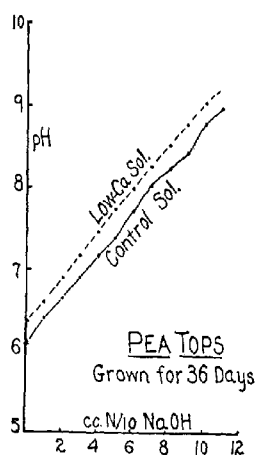
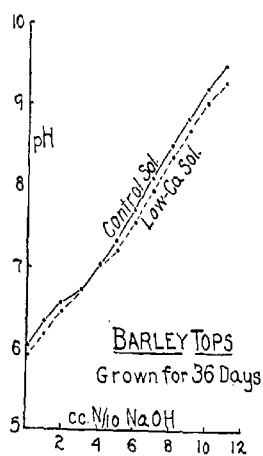
	NUTRIENT SOLUTION	AGE	GREEN WEIGHT PER PLANT	Ca	K	N
<i>Barley and peas grown together</i>						
		days	gm.	p.p.m.	p.p.m.	p.p.m.
Barley top sap.....	Control	51	4.58	855	4300	
	Low calcium	51	4.29	495	4250	
Barley root sap.....	Control	51	1.33	100	3520	
	Low calcium	51	1.46	95	2565	
Pea top sap.....	Control	51	6.29	802	4190	
	Low calcium	51	5.68	395	4190	
Pea root sap.....	Control	51	2.69	230	3260	
	Low calcium	51	2.29	155	3550	
<i>Barley and beans grown together</i>						
Barley top sap.....	Control	56	1.31	850	5817	2240
Bean top sap.....	Control	56	8.28	1305	3830	2220

*Details of the experiment*

The plants for the studies on sap were grown in nutrient solutions in all cases, and in most cases barley and a legume were grown together, as in previously described experiments. The plants were frozen immediately after harvesting. Later they were thawed out, and the expressed juice was filtered on a Büchner funnel, and titrated with the hydrogen electrode. In many cases, samples were analyzed for certain elements (table 9).

an excess of acid, determinations were made of the actual acidities, buffer values, and composition of the juices of barley, peas and beans which had a plentiful supply of calcium, and of others which had had a limited supply of calcium. These experiments with plant sap are intimately related to the previously described absorption experiments, because they are involved in the question of the absorption and function of calcium.

## TITRATION OF 25CC. SAP.



# TITRATION OF 2.5CC. SAP.

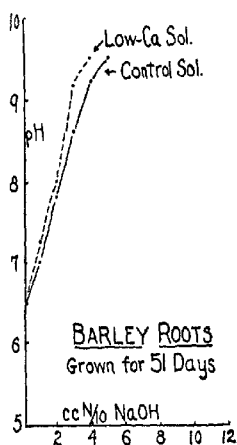


FIG. 5.

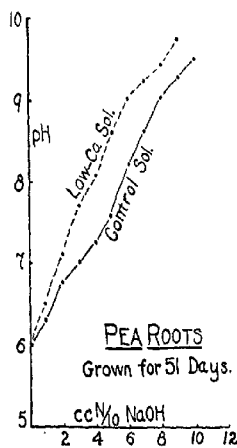


FIG. 6.

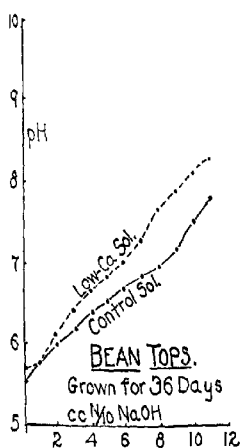


FIG. 7.

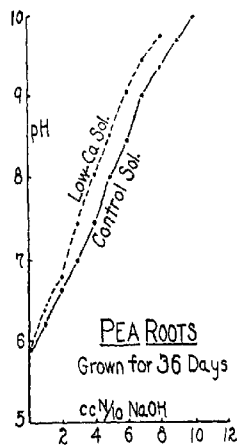


FIG. 8

Strong evidence has been obtained in these experiments that, in the case of barley, peas, and beans, the hydrogen-ion concentration of the plant sap is not appreciably increased by limiting the plant's supply of calcium (figs. 1, 2, 3, 4, 5, 6, 7, 8). The hydrogen-ion concentration was not increased in spite of the fact that analyses proved that there was actually less calcium present in the sap (table 8) and also a less percentage of calcium in the plant, as shown in the earlier experiments.

Though this result may at first seem surprising, it is not difficult to understand why a plant sap containing little calcium might not be any more acid than a plant sap with more calcium. Calcium is not the only base-forming element present in the sap; there is some magnesium and much potassium present also. Salts formed by combinations of these elements with organic acids are undoubtedly present in the sap, and potassium probably replaces calcium to some extent in these organic salts when the ratio of calcium to potassium is decreased, either by an increase in potassium or a decrease in calcium. This might actually produce a more alkaline reaction in the plant sap, because KOH is a stronger base than  $\text{Ca(OH)}_2$ , and potassium salts would hydrolyze to a greater extent than calcium salts. As a matter of fact the tendency in these experiments seemed to be in the direction of slightly greater alkalinity as the concentration of calcium decreased in the sap<sup>3</sup> (fig. 2 and 4).

Truog and Meacham (19), Haas (7), and Clevenger (2) determined the hydrogen-ion concentration of the sap of plants grown on limed and unlimed soil. Truog and Meacham, and Haas concluded that the sap of limed plants had a tendency towards greater alkalinity than that of unlimed plants. In a number of cases, however, they found that the sap of plants grown on limed soil was more acid than the sap of the same plants grown on unlimed soil. Haas suggested that in these cases, the plants had not absorbed as much calcium from the limed as from the unlimed soil, and Clevenger suggested that the increase in lime stimulated the life process of the plant and caused an increased production of organic acids within the plant. Both suggestions would attribute the increase in acidity to lack of calcium. However it was shown in the experiment previously described that a decrease in amount of calcium actually present in plant sap (or in the plant as a whole) does not necessarily accompany an increase in its hydrogen ion concentration.

Even though plant sap is not rendered more acid by lack of calcium, it is still quite possible that this lack of calcium could produce injury by permitting an accumulation of organic acid salts in the sap. The bivalent ion, calcium, would precipitate organic acids more readily than the monovalent ion potassium, and would thus reduce the concentration of organic acid salts present in the sap.

<sup>3</sup> The sap from plants as obtained by the usual methods of expression may not necessarily be identical with the cell sap. It may be questioned whether the reaction of the latter is really subject to appreciable change in a normal plant.

# TITRATION OF 25CC SAP.

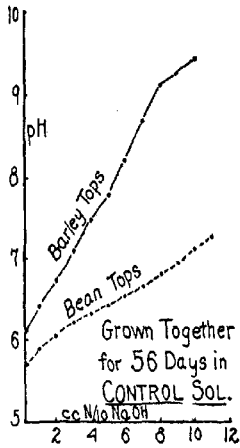


FIG. 9.

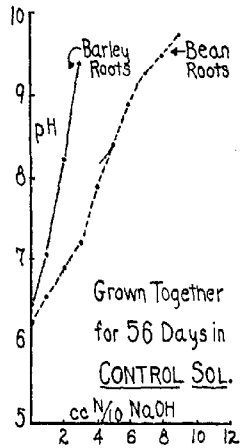


FIG. 10.

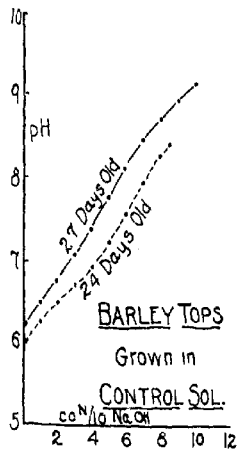


FIG. 11.

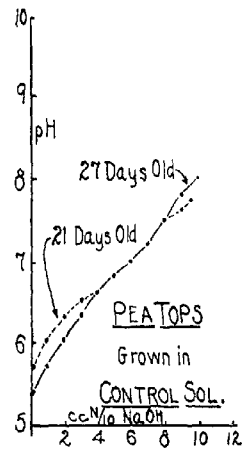


FIG. 12.



Other points which may be noted in the graphs (fig. 1-12) are these:

There was considerable variation in original pH value of the sap of one species, caused, perhaps, by differences in season, age, or other environmental factors.

In most cases the top sap of barley, peas, and beans was more acid than the root sap, but an exception was noted in one experiment with peas, the top sap being more alkaline than the corresponding root sap (fig. 2 and 8).

Barley and pea top juices have approximately equal buffer values (fig. 1 and 2, 3, and 4). Bean top juice has greater buffer value (fig. 9).

Pea and bean root juices have greater buffer values than barley root juice. Pea and bean root juices are much alike in this respect (fig. 5, 6, and 10).

The reactions of the three nutrient solutions used in the different experiments were determined before and after the plants had been grown therein. The original solutions were found to have pH values varying from 4.5 to 5.5, but after the plants had absorbed from the solutions for some time the pH values varied from about 6.8 to 7. Barley, peas and beans grown separately all affected the solutions in this way, the larger plants bringing the solution to this approximately neutral point relatively rapidly. This is in accord with the results obtained by Hoagland, (9), who showed, in his work with barley, that there is a tendency towards the production of a neutral reaction in the nutrient solution, as a result of changes in equilibria caused by absorption.

#### SUMMARY

1. Comparisons, at four different stages of growth, of percentage composition, and of ratios in which ion equivalents were absorbed, brought out no evidence of characteristic differences in the types of absorption from nutrient solutions by barley and peas, or by barley and vetch.

2. Evidence from two experiments indicates that there is a characteristic difference in the type of absorption of barley and beans in the early stages at least. Beans (speckled cranberry) absorb more calcium in proportion to potassium, from a given nutrient solution, than either barley or peas.

3. Evidently the quantity of calcium absorbed by a plant does not necessarily depend upon the quantity of nitrogen absorbed or metabolized, as has been suggested by other workers. It was found possible to decrease the quantity of calcium absorbed by barley and peas without decreasing the quantity of nitrogen absorbed, by growing the plants in a solution low in calcium. Moreover, it was found that inoculated peas absorbed more nitrogen than uninoculated, when grown in the cultures low in nitrogen, but did not absorb more calcium, though there was plenty of this element available.

4. Peas were found to contain a much higher percentage of calcium than barley, and a considerably higher percentage of magnesium when they were grown side by side in an adobe soil. The differences in percentage of potas-

sium, nitrogen, and phosphorous were smaller, but all were in favor of the peas. Vetch, like peas, was found to have a much higher percentage of calcium than barley, when grown in a soil, but not when grown in a nutrient solution.

5. The quantity of carbon dioxide given off from the roots of pea plants grown in sand cultures was found to be very much greater than that given off from the roots of barley plants of a similar size. This offers a very probable explanation for the fact that pea plants absorb more calcium and magnesium from a soil than barley plants, although they do not absorb more calcium or magnesium from a nutrient solution. In the soil, carbon dioxide would affect the solubility of calcium and magnesium compounds.

6. Evidence was obtained in the case of barley, peas and beans, that the hydrogen-ion concentration of the plant sap was not appreciably increased by limiting the plant's supply of calcium. Analyses proved in these cases that there was actually less calcium present in the sap, and a less percentage in the plant as a whole, as indicated by previous experiments. The buffer effect of barley and pea sap was similar, but was greater in the case of beans.

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## SULFUR, CALCIUM, MAGNESIUM AND POTASSIUM CONTENT AND REACTION OF RAINFALL AT DIFFERENT POINTS IN TENNESSEE

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The amount of sulfur precipitated annually at the University of Tennessee Experiment Station Farm has been determined since 1913 in connection with certain lysimeter investigations. The point of collection is located about  $1\frac{1}{2}$  miles from the center of Knoxville, a city of extensive manufacturing interests and of a population close to 100,000. From 1913 to 1916 only the total amount for the year was determined. Since January 1917, however, each quarterly precipitation also has been determined. Beginning January 1915, the sulfur precipitation at the Weather Bureau Observatory in the heart of the city has been determined both in annual and quarterly parallels with those at the University Farm. Starting with the fourth quarter of 1918, quarterly and annual determinations have been made also on the rain water collected on a farm near Knoxville and on collections from seven other points in the State. The sulfur precipitations at the University Farm from 1913 to 1922, those at the Knoxville Weather Bureau Observatory from 1915 to 1922 and also the 3-year results at the other Knox County farm are given in table 1. The sulfur data for the seven other points for the years 1919, 1920, and 1921 are given in table 2. These findings have added interest when compared with earlier records contributed by Crowther and Ruston (1), Crowther and Stewart (2), Miller (6), Stewart (8), and more recently (9) by Wilson.

### SAMPLING AND ANALYSIS

The samples were collected in standard copper rain gauges or in asphaltum-painted galvanized receptacles of the same dimensions and transferred to glass containers after each rainfall. The clear supernatant rain water was syphoned off from the settled soot and other extraneous materials, or if necessary these were removed by filtration at the time of making the analyses. After concentration, iron and aluminum were removed by ammoniacal precipitation, and calcium and magnesium were determined by the standard methods. The use of Nessler's reagent demonstrated that only fractions of one part per million of nitrogen, as  $\text{NH}_3$ , were ever present; while the same finding, with reference to nitrate nitrogen, was obtained by the phenol-disulfonic acid procedure. Silica was never present in amounts sufficient to necessitate its preliminary removal in any of the analytical procedures except in the case of total salts converted to sulfates. Repeated sulfate determinations, with and without preliminary oxidation, established the fact that all soluble sulfur was present as sulfates. Sulfate determinations were made in duplicate, from concentrates equivalent to either 1 or 2 liters

of the original rain water. Total possible sulfates were determined by evaporating the equivalent of either one or two liters of the original rain water with an excess of  $\text{H}_2\text{SO}_4$ , dissipation of the excess of  $\text{SO}_3$ , dissolving of the residual sulfates with  $\text{HCl}$ , and precipitation as  $\text{BaSO}_4$ . Chlorine was determined by titration with silver nitrate in concentrates equivalent to 2 liters of the original rain water. Phosphates were determined by the A. O. A. C. official volumetric method upon 2-liter concentrates. Potassium was determined as  $\text{K}_2\text{PtCl}_6$  after the elimination of the trace of ammonia. The reactions were determined quantitatively upon either 100-cc. or 200-cc. portions by titration with 0.05 N acid against both methyl orange and cochineal.

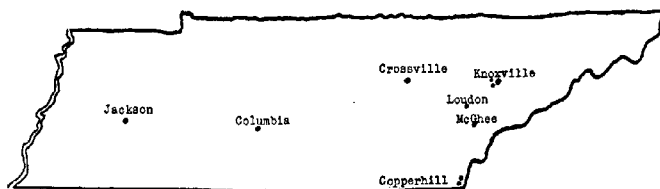


FIG. 1. MAP OF THE STATE OF TENNESSEE, SHOWING THE LOCATIONS OF THE TEN SAMPLING POINTS AT WHICH RAINFALL WAS COLLECTED IN STANDARD 8-INCH GAUGES

#### SULFUR PRECIPITATIONS

*Knoxville, University Farm.* The first three years gave 68.7 lbs., 50.6 lbs. and 77.8 lbs. of sulfur per acre surface. The amounts for the first and third years are somewhat above the rather closely agreeing results for the years 1917–1921. The 8-year average of 51.5 lbs. represents non-acid sulfate sulfur. Such an average precipitation of sulfur would be equivalent to an assumed combination of 218.6 lbs. of  $\text{CaSO}_4$ . The data show that the sulfur precipitation came heaviest during the winter; that is, in the first and fourth periods. The third quarter yielded the lightest amount in four of the five years of quarterly determinations, the difference being but slightly greater for the third than for the second quarter, in the fifth instance. The sulfur content of the rainfall thus ran parallel with the consumption of soft coal.

*Knoxville, Weather Bureau Observatory.* The greater amount of soluble sulfate sulfur precipitated within the city is most noticeable for the 1918–1922 period. The total of 175.1 lbs. of sulfur during 1918 causes this year to stand out as abnormal, especially in the first, third, and fourth quarters. The result for the fourth quarter of 1919 was obtained by averaging the data for the corresponding period of the years 1917, 1918, 1920 and 1921, because of the loss of the quarterly sample overnight by freezing. The block of ice saved from this freezing was about one-eighth of the volume of the original sample. It was melted and analyzed for sulfates, yielding 0.3496 gm. of  $\text{BaSO}_4$  per liter. While it is possible that this abnormal concentration may represent the composition of the original water, it is more probable that the freezing was so gradual as to effect a concentration of salts in the core represented by the block of ice found on the floor near the broken bottle the following morning.

TABLE 1  
Quarterly and annual rainfall and sulfur precipitated at three points in Knox County, Tennessee

YEAR	FIRST QUARTER			SECOND QUARTER			THIRD QUARTER			FOURTH QUARTER			ANNUAL		
	Rainfall	BaSO <sub>4</sub> per liter	S per acre	Rainfall	BaSO <sub>4</sub> per liter	S per acre	Rainfall	BaSO <sub>4</sub> per liter	S per acre	Rainfall	BaSO <sub>4</sub> per liter	S per acre	Rainfall	BaSO <sub>4</sub> per liter	S per acre
Knoxville, Knox Co., University Farm. Altitude 900 ft.															
1913*	21.58	.....	.....	9.42	.....	.....	9.01	.....	.....	7.36	.....	.....	47.37	.0443*	68.7*
1914*	10.28	.....	.....	8.83	.....	.....	12.68	.....	.....	12.18	.....	.....	43.97	.0412*	50.6*
1915*	11.50	.....	.....	10.56	.....	.....	15.38	.....	.....	16.74	.....	.....	54.18	.0505*	77.8*
1916*	13.61	.....	.....	13.36	.....	.....	14.49	.....	.....	9.33	.....	.....	50.79	.....	.....
1917	20.09	0.0227	14.2	12.26	0.0233	8.9	13.74	0.0138	5.9	5.42	0.0470	14.6	51.51	.....	43.6
1918	11.40	0.0382	13.6	13.73	0.0184	7.9	10.01	0.0272	8.5	11.24	0.0429	15.0	46.38	.....	45.0
1919	16.01	0.0385	19.2	13.82	0.0220	9.5	9.29	0.0240	6.9	18.53	0.0272	8.5	57.65	.....	44.1
1920	13.79	0.0360	15.4	16.79	0.0186	9.7	17.56	0.0130	7.1	10.48	0.0362	11.8	58.62	.....	44.0
1921	11.78	0.0392	12.9	9.10	0.0288	9.0	12.76	0.0162	5.0	9.67	0.0356	11.1	43.31	.....	38.0
Average	14.45	0.0349	15.1	11.99	0.0222	9.0	12.77	0.0188	6.7	11.22	0.0378	12.2	50.42	.....	51.5
Knoxville, Knox Co., Weather Bureau Observatory. Altitude 900 ft.															
1915	10.12	.....	.....	11.28	.....	.....	11.71	.....	.....	15.93	.....	.....	49.04	.....	79.1
1916	10.81	.....	.....	12.03	.....	.....	16.01	.....	.....	8.15	.....	.....	44.55	.....	67.1
1917	23.70	0.0533	39.3	10.14	0.0420	13.2	22.85	0.0277	19.7	5.39	0.1426	23.9	59.72	.....	96.1
1918	10.42	0.0216	65.3	15.02	0.0546	25.5	9.36	0.1263	36.8	12.50	0.1221	47.5	43.79	.....	175.1
1919	12.30	0.0662	25.3	13.33	0.0600	24.9	13.30	0.0352	14.6	11.95	†	27.1	46.30	.....	91.9
1920	12.77	0.0724	28.8	16.71	0.0570	29.6	20.70	0.0170	11.0	9.70	0.752	22.7	55.21	.....	92.1
1921	11.66	0.0642	23.3	8.08	0.0392	12.2	13.75	0.0236	10.1	8.40	0.0462	14.4	40.45	.....	60.0
Average	13.11	0.0915	36.4	12.37	0.0506	21.1	15.38	0.0460	18.4	10.29	0.0965	27.7	48.44	.....	94.5
7 mi. from Knoxville, Knox Co., Ogden Farm. Altitude 900 ft.															
1919	16.01	0.0102	5.1	13.82	0.0136	5.8	9.29	0.0112	3.2	18.53	0.0082	4.7	57.65	.....	18.8
1920	13.79	0.0060	2.6	16.79	0.0150	7.8	17.56	.....	3.2	10.48	.....	4.7	58.62	.....	18.3
Average	14.90	0.0081	3.9	15.31	0.0143	6.8	13.43	0.0112	3.2	14.51	0.0082	4.7	58.14	.....	18.6

\* Annual Rainfall collections November to November calculated on same basis.

† Frozen.

TABLE 2  
*Quarterly and annual rainfall and sulfur precipitated at seven points in Tennessee*

YEAR	FIRST QUARTER				SECOND QUARTER				THIRD QUARTER				FOURTH QUARTER				ANNUAL		
	Rainfall	BaSO <sub>4</sub> per liter	S per acre		Rainfall	BaSO <sub>4</sub> per liter	S per acre		Rainfall	BaSO <sub>4</sub> per liter	S per acre		Rainfall	BaSO <sub>4</sub> per liter	S per acre		BaSO <sub>4</sub> per liter	S per acre	
Copperhill, Weather Bureau Observatory, Polk, Co. Altitude 1600 ft.																			
1919	17.76	0.0196	10.8	in.	13.11	0.0428	17.4	lbs.	12.39	0.0884	34.1	lbs.	10.33	0.1614	50.2	lbs.	53.59	.....	112.5
1920	16.93	*	10.8	in.	18.84	0.0396	23.2	lbs.	21.79	0.0488	67.8	lbs.	14.29	0.1444	64.2	lbs.	71.80	.....	166.0
1921	13.33	0.1998	82.8	in.	10.87	0.1408	47.6	lbs.	15.01	0.1602	74.8	lbs.	14.43	0.4760	213.6	lbs.	53.64	.....	418.8
Average	16.01	0.1097	34.8	in.	14.27	0.0744	29.4	lbs.	16.40	0.0991	58.9	lbs.	13.02	0.2606	109.3	lbs.	59.68	.....	232.4
6 mi. from Copperhill, Polk Co. Altitude 1600 ft.																			
1919	17.76	0.0705	38.9	in.	13.11	0.0176	7.2	lbs.	12.39	0.0192	7.4	lbs.	10.33	0.0358	11.5	lbs.	53.59	.....	65.0
1920	16.93	0.0188	9.9	in.	18.84	0.0584	34.2	lbs.	21.79	0.0230	15.6	lbs.	14.29	0.0246	10.9	lbs.	71.80	.....	70.6
1921	13.33	0.0334	13.8	in.	10.87	0.0036	1.2	lbs.	15.01	0.0568	26.5	lbs.	14.43	0.0878	39.4	lbs.	53.64	.....	80.9
Average	16.01	0.0409	20.9	in.	14.27	0.0265	14.2	lbs.	16.40	0.0330	16.5	lbs.	13.02	0.0494	20.6	lbs.	59.68	.....	72.2
McGee, Monroe Co. Altitude 850 ft.																			
1919	15.44	0.0182	8.7	in.	9.86	0.0188	5.8	lbs.	6.70	0.0164	3.4	lbs.	12.52	*	5.1	lbs.	44.52	.....	23.0
1920	15.42	0.0190	9.1	in.	16.05	0.0104	5.2	lbs.	21.27	0.0082	5.4	lbs.	12.21	0.0182	6.9	lbs.	64.95	.....	26.6
1921	13.98	0.0228	9.9	in.	10.73	0.0108	3.6	lbs.	13.02	0.0150	6.1	lbs.	10.76	0.0098	3.3	lbs.	48.49	.....	22.9
Average	14.95	0.0200	9.2	in.	12.21	0.0133	4.9	lbs.	13.66	0.0132	5.0	lbs.	11.83	0.0140	5.1	lbs.	52.65	.....	24.2

Louden, Louden Co. Altitude 750 ft.

1919	17.97	0.0124	7.0	10.23	0.0120	3.8	6.93	0.0078	1.7	16.38	*	3.6	51.51	.....	16.1
1920	14.59	0.0146	6.6	17.24	0.0082	4.4	20.11	0.0130	8.1	12.97	0.0068	2.7	64.91	.....	21.8
1921	13.69	0.0182	7.4	10.61	0.0116	3.8	16.40	0.0084	4.3	10.38	0.0138	4.5	51.08	.....	20.0
Average	15.42	0.0151	7.0	12.69	0.0106	4.0	14.48	0.0097	4.7	13.24	0.0103	3.6	55.83	.....	19.3

Crossville, Cumberland Co. Altitude 1850 ft.

1919	13.41	0.0036	1.5	10.27	0.0150	4.8	7.57	0.0036	0.8	16.05	0.0058	2.9	47.30	.....	10.0
1920	17.35	0.0102	5.5	16.34	0.0086	4.4	25.10	0.0018	1.4	10.20	0.0094	3.0	68.99	.....	14.3
1921	16.01	0.0092	4.6	13.96	0.0094	4.1	14.51	0.0062	2.8	8.98	0.0082	2.3	53.46	.....	13.8
Average	15.59	0.0077	3.9	13.52	0.0110	4.4	15.73	0.0039	1.7	11.74	0.0078	3.1	56.58	.....	12.7

Columbia, Maury Co. Altitude 725 ft.

1919	16.55	0.0167	8.6	16.50	0.0106	5.4	10.20	0.0108	3.4	22.55	0.0142	10.0	65.80	.....	27.4
1920	15.50	0.0188	5.8	21.39	0.0082	5.5	12.70	0.0076	3.0	7.75	0.0326	7.9	57.34	.....	22.2
1921	14.33	0.0037	1.6	9.70	0.0158	4.8	17.00	0.0270	14.3	10.70	0.0264	8.8	51.73	.....	29.5
Average	15.46	0.0131	5.3	15.86	0.0115	5.2	13.30	0.0151	6.9	13.67	0.0244	8.9	58.29	.....	26.4

Jackson, Madison Co. Altitude 440 ft.

1919	17.64	0.0382	31.9	11.00	0.0376	12.9	8.34	0.0486	12.6	22.09	0.0230	15.8	59.07	.....	73.2
1920	13.54	0.0526	22.2	13.01	0.0378	15.3	9.18	0.0300	8.6	7.18	0.0438	9.8	42.91	.....	55.9
1921	15.85	0.0398	19.6	10.75	0.0256	8.6	16.01	0.0212	10.6	7.61	.....	9.8	50.22	.....	48.6
Average	15.68	0.0502	24.6	11.59	0.0337	12.3	11.18	0.0333	10.6	12.29	0.0334	11.8	50.73	.....	59.2

\* Broken in transit.



If the decidedly abnormal sulfate content of the melted ice were taken as representative, the sulfur precipitation for the fourth quarter of 1919 would appear as 129.9 pounds per acre. This, added to the determined amount of the first three quarters, would give a total precipitation of 194.7 pounds, rather than the reported occurrence of 91.9 pounds for the 1919 annual period. The average of the 7-year period, including the 1919 result above explained, amounts to 94.5 pounds of soluble sulfate sulfur per acre as compared to that of 51.5 pounds as the 8-year average secured at the suburban University Farm.

It has not been generally conceded that fuel consumption is the absolute, or even the paramount, cause of the presence of sulfur in the atmosphere. Lawes, Gilbert and Warrington (4) state: "The sulfates of the atmosphere are, according to Angus Smith, chiefly derived from the oxidation of sulphur compounds evolved during the decay of animal matter." And again the same authors (5) state: "The considerable amount of sulphuric acid found in the summer rain points, however, to a further source, most active during high temperatures. This indication is quite in accordance with the opinion held by Dr. Angus Smith, that the sulphuric acid in the rain is largely derived from the products of decay of animal and vegetable matter, which is of course most active in the warmer months." These authors took Dr. Smith's statement from his book *Air and Rain; the Beginning of Chemical Climatology* which was published in 1872.

*Ogden Farm, near Knoxville.* On this farm, located about 7 miles west of Knoxville, the collections were made by the owner and because of the sale of the property were discontinued after seven quarters. The total amount of sulfur in the rain water was decidedly less than that at the University Farm, which in turn was less than that found in the heart of the city. The Ogden Farm rain water carried very little soot. Thus, with increasing distance from the source of the soot and other solid matter, the occurrence of sulfur was consistently less. This indicates that the major portion of the sulfate sulfur precipitation may be carried by the solid matter rather than as dissolved gases.

*Copperhill Weather Observatory.* Official rainfall collections were secured from this point, which has an elevation of 1600 feet. The town is the center of an extensive copper industry. In past years vast quantities of sulfur were given off by the open burning of the copper ores and the non-recovery of the evolved sulfur. Extensive areas were devastated of vegetation as a result of the discharge of the acid fumes. Since the enactment of laws necessitating the installation of an acid plant, the evolutions of sulfur have been reduced to the minimum. The data show that the correlation between the domestic or seasonal coal consumption and sulfur precipitation is not so distinct as in Knoxville, a coal burning commercial center. The sample for the first quarter of 1920 was broken in transit and the corresponding figure of 1919 was used in obtaining the total for the annual period. An abnormal discharge occurred during the year 1921 on account of curtailment in the use of transmitted

current for power and the substitution of power generated locally by several large boilers in the Copperhill smelters. For that year there was found a precipitation of 418.8 pounds of sulfur per acre, enhancing the 3-year average to 232.4 pounds.

*Near Copperhill, Weather Bureau Observatory.* The sampling point is located about six miles distant from Copperhill and it was expected that the sulfur brought down at this location would throw some light upon the aerial diffusion of the somewhat unusual smelter "escapages," at Copperhill. The average of 72.2 pounds for the years 1919, 1920 and 1921 is about half way between the average of the Knoxville Weather Bureau Observatory and that of the University Farm.

*McGhee Weather Observatory.* This station is about 35 miles southwest of Knoxville and about 65 miles from Copperhill, and has an altitude of 850 feet. It was included along with Loudon, in order to demonstrate, if possible, any relationship between the source of an extensive discharge of sulfur and the direction of the prevailing winds. The town has a population of about 200. The McGhee sample for the fourth quarter of 1919 was broken in transit, and the average of 5.1 pounds for the fourth quarters of 1920 and 1921 was therefore used in obtaining the total for the year 1919. The average of 24.2 pounds for the three-year period is less than half that found at the University Farm. Though none of the quarterly amounts at this station was large, those of the January-March period were consistently higher than those of the other three quarters.

*Loudon Observatory.* This station is also a small town, of about 1800 inhabitants. It is located about 50 miles from the ore-roasting plant at Copperhill, and has an elevation of 750 feet. The Loudon sample for the fourth quarter of 1919 was likewise broken in transit, and the average figure was inserted in obtaining the total for the year, as in the case of the same period for McGhee. The average annual precipitation of 19.3 pounds per acre was even less than that at McGhee.

*Crossville Farm.* The samples from this location were collected by one of the coöperative assistants of the University, at a point about six miles from the small town of Crossville. Crossville is located upon the Cumberland Plateau, a sparsely inhabited section and has an altitude of 1850 feet. The amounts of sulfur washed down at this point were the smallest of all of the ten locations. The average for the three years amounts to but 12.7 pounds per acre. The differences between quarterly amounts are not of such magnitude nor consistency as to warrant special stress.

*Columbia Station Farm.* This source of sampling is located upon the Middle Tennessee Experiment Station Farm, about two miles from the city of Columbia. A side-line railroad passes through the farm. A quarterly comparison does not show any striking relationship between season and the amounts of sulfur brought down. The smallest amount happens to be credited to the

first quarter of 1921, while the largest amount was obtained in the period of July, August, and September of the same year. The average for the 3-year period amounts to 26.4 pounds of sulfur per acre.

*Jackson Station Farm.* The collections made at this point were secured from the West Tennessee Experiment Station, about two miles from the city of Jackson, in the Mississippi Valley. A branch-line railroad passes through this farm also. The average for the first quarter of each of the three years is about twice that of the average of each of the other three quarters. These latter averages are rather consistent. The annual average for the three years shows a precipitation of 59.2 pounds per acre. It was rather surprising to find so high an average—one greater than that of the University Farm, adjacent to Knoxville—especially since the prevailing winds are not from the town toward the station.

#### REACTION OF THE RAIN WATER

Each sample of rainfall was examined as to its reaction to common indicators. The averages of titrations for each quarter for the several years are given in table 3. The figures represent cubic centimeters of 0.05 *N* acid required to neutralize a liter of the rain water, both methyl orange and cochineal having been used as indicators. The titrations against methyl orange are consistently higher than those made with cochineal in all periods and for all sampling points. It was anticipated that the amounts of  $\text{SO}_2$  evolved from the combustion of soft coal used throughout the area under observation would produce an acid-reacting rain water. However, such did not prove to be the case. In the 320 titrations made in duplicate, not one failed to show alkaline toward methyl orange, while the same was true also, except in three instances, where cochineal was used. In one of these three cases neutrality was shown to cochineal, while a mere trace of acidity was found in the other two instances.

The dry combustion of bituminous coal produces  $\text{SO}_2$ , which may be readily detected under conducive atmospheric conditions. The ash, however, both residual and evolved, is distinctly alkaline from the presence of alkali and alkali-earth combinations. Nevertheless, the prevailing conditions of fuel combustion are such as to preclude the neutralization of the volatile acid gas by the preponderance of dry alkali and alkali-earth bases until the solid and gaseous phases are brought into intimate contact through solution in the rainfall, either during precipitation, or after collection in the gauges.

In searching for parallel reaction determinations, we have found only the results of Crowther and Ruston (1). These workers report free acidity amounting to 20.05 pounds of  $\text{H}_2\text{SO}_4$  as an average annual precipitation near Leeds during the years 1906-1909. The rain water acidity reached the high mark of 41.4 parts per million during the foggy month of December, 1908. Methyl orange was used as an indicator against 0.01*N* alkali. They conclude: "The results bear unmistakable evidence of the fact that the impurities have their origin largely in the combustion of coal, this being the most obvious source of the acid which occurs in such appreciable quantities in the rain." On the other hand, in the same paper the findings at Leeds are shown to have been entirely different. At this point a total of 1886

TABLE 3  
*Alkalinity determinations, as averages of 1919, 1920 and 1921 rainfalls, collected at 10 different points—titrations with 0.05 N HCl—methyl orange and cochineal as indicators*

QUARTERS	KNOXVILLE WEATHER BUREAU			UNIVERSITY FARM			NEAR KNOXVILLE			COPPER HILL			NEAR COPPER HILL			LOUDON			MCGHEE			CROSSVILLE			COLUMBIA			JACKSON		
	0.05 N acid			0.05 N acid			0.05 N acid			0.05 N acid			0.05 N acid			0.05 N acid			0.05 N acid			0.05 N acid			0.05 N acid			0.05 N acid		
	Methyl orange	Cocheineal	cc.	Methyl orange	Cocheineal	cc.	Methyl orange	Cocheineal	cc.	Methyl orange	Cocheineal	cc.	Methyl orange	Cocheineal	cc.	Methyl orange	Cocheineal	cc.	Methyl orange	Cocheineal	cc.	Methyl orange	Cocheineal	cc.	Methyl orange	Cocheineal	cc.	Methyl orange	Cocheineal	cc.
First.....	3.35	1.35	5.00	2.65	3.50	3.00	4.00	1.75	4.50	1.00	8.15	4.35	7.85	4.35	7.15	3.65	4.65	1.25	5.50	2.50	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
Second.....	3.85	1.50	4.85	3.85	6.00	3.75	4.75	2.75	9.15	5.70	4.85	2.35	6.15	3.35	5.35	2.50	3.35	1.00	2.85	1.15	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
Third.....	3.85	1.50	5.65	3.35	2.00	0	5.85	3.50	13.85	10.15	7.15	3.85	5.85	3.85	3.65	0.85	5.35	2.15	3.65	1.35	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
Fourth.....	2.50	0.75	4.35	2.15	2.00	0	40.00	17.05	27.00	22.15	6.00	3.50	4.25	1.25	4.35	0.85	5.00	1.25	5.50	3.25	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
Average for annuals.....	3.39	1.28	4.96	3.00	3.38	1.69	13.65	6.26	13.63	9.75	6.54	3.51	6.03	3.20	5.13	1.96	4.59	1.41	4.38	2.06	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.

pounds of suspended matter fell upon an acre surface during the year, and of this amount 1113 pounds was ash. The authors state, "At this station (Leeds Forge), indeed, the rain was in some cases appreciably alkaline in reaction."

Later, Crowther and Stewart (2), offer findings obtained at 14 stations within a short radius of Leeds. Of 153 samples collected at seven different points 140 were either neutral or distinctly alkaline to methyl orange, congo red, and litmus, while the remaining 13 samples were acid. Finding but a fraction of the total sulfur in unoxidized form, an alkaline rainfall, and citing the conclusions of Herbig (3) to the effect that not more than one-hundredth of the volatilized sulfur leaves the chimney as  $\text{SO}_2$ , Crowther and Stewart (2) offer the conclusion: "It is possible that, contrary to the commonly held opinion, the smoke arising from the combustion of coal under industrial conditions may in some cases contain its sulfur not mainly as sulfur dioxide, but almost entirely in the form of sulfate."

According to Richards (7), "Rainwater is very nearly saturated with oxygen when its temperature as collected is below  $15^\circ\text{C}$ ," and, "When the temperature of the rain is above  $15^\circ\text{C}$ , the dissolved oxygen is always below saturation, occasionally as much as 25 per cent." In addition, the tendency of finely divided base-impregnated soot and prevalent dust to absorb gases affords further oxidizing and neutralizing possibilities. Oxidation may also be accounted for, according to Lawes, Gilbert and Warrington (4), by atmospheric hydrogen peroxide, and since this compound "is evolved when turpentine, and possibly other bodies, are oxidized in the air, the neighborhood of a pine forest should be favorable to the formation of nitric acid" (and sulfates)<sup>1</sup> "in the atmosphere." As stated in description of methods of analysis, we found no unoxidized sulfur dissolved in the cumulative samples at the time of analysis. Thus, in the case of the Tennessee precipitations, the rapid diffusion of  $\text{SO}_2$ , the excess of solid alkaline material in the smoke, and aerial oxidizing agencies were apparently responsible for complete oxidization of the soluble sulfurous content of rainwaters of alkaline reaction. This is in decided contrast to the marine-influenced rainfall found in England, where a maximum parallel of 380 pounds of  $\text{SO}_2$  and 70 pounds of chlorine and a minimum parallel of 128 pounds of  $\text{SO}_2$  and 40 pounds of chlorine per acre per annum, with about one-fourth of the total sulfur "in other forms oxidizable to sulfate."

#### CALCIUM AND MAGNESIUM IN RAINFALL

Little attention seems to have been paid to the basic-element content of rainfall. Lawes, Gilbert and Warrington (4) found but 4.0 parts per million of hardness in a total solid-matter content of 28.0 parts per million, as determined upon 22 samples "from washed gauge" at Rothamsted. "By 'hardness' is understood the total lime and magnesia in water, expressed in parts of carbonate of calcium." Analysis of a composite collection of 47 samples of rainwater from the unwashed gauge gave a total hardness of 4.8 parts per million and a total-solids content of 36.6 parts per million. Again, from 7 samples of dew and hoar-frost the same authors found an average of 19.0 parts per million in an average total-solids occurrence of 48.7 parts per million. Miller (6) compiled an extensive bibliography of 125 references to rainfall data in which Marggraf is cited as having found lime along with nitric acid and chlorine in 1749-1750, confirmed by Bergman, in both rainfall and snow.

It was known that  $\text{SO}_2$  is given off at both Knoxville sampling points as a result of the combustion of soft coal. As previously stated, such a discharge may be detected readily by its odor, at times when conducive atmospheric conditions prevail. Consequently when it was found that the rain waters were not acid and that lime and magnesia were present in considerable quantities, further information was sought.

Seven of the quarterly collections from the Knoxville Weather Bureau Observatory and six of those of corresponding periods from the University

<sup>1</sup> (and sulfates) inserted by authors.

Farm were analyzed for lime and magnesia. The amounts of CaO and MgO found, in terms of  $\text{BaSO}_4$ , and also the amounts of  $\text{BaSO}_4$  found in the determinations of total sulfates, are given in table 4.

As previously stated, it seemed possible that the evolved acid gas might be oxidized by aeration and by atmospheric ozone and hydrogen peroxide, bringing the dissolved oxidized sulfur into contact with small particles of limestone dust and basic silicates which would function as neutral-

TABLE 4  
*Sulfates accounted for in rainfall by direct determination of calcium and magnesium and assignment of difference to alkali sulfates\**

YEAR	COLLECTION PERIOD	WHERE COLLECTED	TOTAL $\text{BaSO}_4$ DETERMINED PER LITER	$\text{BaSO}_4$ EQUIVALENT OF DETERMINED CaO PER LITER	$\text{BaSO}_4$ EQUIVALENT OF DETERMINED MgO PER LITER	$\text{BaSO}_4$ PER LITER UNACCOUNTED FOR BY CALCIUM AND MAGNESIUM SULFATES	PER CENT OF DETERMINED SULFATES ASSIGNED BY DIFFERENCE TO ALKALI SULFATES*
			gm.	gm.	gm.	gm.	per cent
1917	April-June	Knoxville W. B.	0.0420	0.0271	0.0093	0.0056	13.3
1917	July-Sept.	Knoxville W. B.	0.0277	0.0121	0.0064	0.0092	33.2
		University Farm	0.0138	0.0029	0.0064	0.0045	32.6
1917	Oct.-Dec.	Knoxville W. B.	0.1426	0.0478	0.0162	0.0786	55.1
		University Farm	0.0470	0.0154	0.0052	0.0264	56.1
1918	Jan.-March	Knoxville W. B.	0.2016	0.0391	0.0145	0.1480	73.4
		University Farm	0.0382	0.0087	0.0075	0.0220	57.6
1918	July-Sept.	Knoxville W. B.	0.1243	0.0233	0.0075	0.0935	75.2
		University Farm	0.0272	0.0108	0.0087	0.0077	28.3
1918	Oct.-Dec.	Knoxville W. B.	0.1221	0.0208	0.0197	0.0816	66.8
		University Farm	0.0429	0.0121	0.0174	0.0134	31.2
1919	Jan.-March	Knoxville W. B.	0.0662	0.0150	0.0208	0.0304	45.9
		University Farm	0.0385	0.0100	0.0156	0.0129	33.5
Average of all determinations. ....			0.0719	0.0196	0.0119	0.0411	46.3

\* Disregarding determined traces of Al, Fe and  $\text{NH}_4$

izing nuclei in the formation of rain drops. It is undoubtedly true that such nuclei are to be found in the atmosphere because of the evolution of ash and because of the use of limestone in the construction of suburban roads. If such an explanation were to be accepted, our calcium and magnesium results would lead to the conclusion, that limestone or dolomitic limestone dust particles were always present in amounts greater than those necessary to neutralize the evolved acid. Furthermore, the excess of alkali-earth dust particles above the amount combined with the evolved acid would be washed

down mechanically. The filtered and unfiltered rainfall carried the same amounts of lime and magnesia, indicating that all soluble calcium and magnesium salts were dissolved either during the precipitation or as a result of the suspension of the soot in rain water during the quarterly period of collection. It was thought that the suburban farm precipitation, which was lower than the city collection in sulfates, would run higher in total lime since the sampling point was adjacent to limestone roads and closer to the road level. But with the increasing amount of sulfates in the heart of the city where the streets are asphalted, and where the collection point was more than a hundred feet above the road level, increased amounts of dissolved neutral calcium and magnesium salts were found. That is, coincident with sulfate increases, there also occurred a distinct increase in dissolved alkali-earth bases.

The average of the  $\text{BaSO}_4$  equivalents of determined  $\text{CaO}$ , table 4, represents 26.1 per cent of the total sulfates. In nine of the 13 samples, the amounts of lime found were greater than the amounts of magnesia present, in terms of chemical equivalence. The average amount of magnesia found in the 13 quarters accounts for 16.6 per cent of the corresponding average for the determinations of the sulfate radical. It is evident, therefore, that a large part of the precipitated sulfate radical occurs in combination with the alkali-earth bases.

In this connection, the washing of the atmosphere by the rainfall at the Knoxville Observatory was studied for the 3-month period of April, May and June, 1918. The total composite precipitation of 15.02 inches during the quarter yielded 0.0546 gm. of  $\text{BaSO}_4$  per liter. In addition to this collection the first  $\frac{1}{4}$ -inch and the second  $\frac{1}{4}$ -inch of each daytime precipitation were collected and analyzed separately, without including the soot which was more abundant in the first fractional composite. The first  $\frac{1}{4}$ -inch yielded 0.2262 gm.  $\text{BaSO}_4$  per liter; the second  $\frac{1}{4}$ -inch, only 0.0902 gm. While it might be contended that this difference was due to the fact that the evolved sulfurous gases had been washed from the atmosphere before they had diffused to the higher altitudes, the same reasoning would also apply to the evolved sulfate-impregnated solid matter of the smoke.

A somewhat related finding, that of Frankland (Sixth Report of the River's Pollution Commission, 1874), has been reported by Lawes, Gilbert and Warrington (4). Anticipating rain, the gauge was cleansed at 11:30 a.m. and the collections made at 3 p.m. and 4:30 p.m. A total solid-matter content of 40.8 parts per million was found in the first sampling, while but 29.4 parts were found in the second collection. The total nitrogen decreased from 1.43 parts per million to 0.69, while the chlorine decreased only from 1.0 part to 0.8 part per million.

It seems logical to conclude, therefore, that the amounts of soluble lime, magnesia and sulfates found in rain waters are determined by their mechanical diffusion to the atmosphere, as components of soot and fine dust.

DIRECT  $\text{BaSO}_4$  DETERMINATIONS AS COMPARED WITH TOTAL POSSIBLE  
SULFATES

During the years 1919 and 1920 the direct  $\text{BaSO}_4$  determinations upon the collections from the 10 sampling points were compared with those determined after all salts were converted to sulfates. An excess of  $\text{H}_2\text{SO}_4$  was added to 1- or 2-liter portions, which were then evaporated and ignited. The residue was taken up with  $\text{HCl}$ , filtered, and the increased combined sulfates determined as  $\text{BaSO}_4$ . The direct sulfate determinations and those found subsequent to the sulfate conversions are given in table 5. In every case the  $\text{BaSO}_4$ -equivalent, after sulfate conversion, is greater than the corresponding direct barium sulfate determination. An average of 0.0341 gm. of  $\text{BaSO}_4$  equivalents is obtained from 36 direct determinations upon the rainfall concentrates, as against 0.0951 gm., representing a similar average for total possible sulfates. These determinations show a rather wide range of proportions between direct and possible sulfates in the several samples, and indicate the occurrence of molar combinations between bases and acid radicals of less combining weight than that of  $\text{SO}_4$ . The amounts of carbonates or bicarbonates indicated by the titrations could not account for this difference, assuming all alkalinity as attributable to non-volatile bases.

## OCCURRENCE OF CHLORIDES AND PHOSPHATES

From comparisons between the total possible sulfates as  $\text{BaSO}_4$ , and the summation of the calculated barium sulfate equivalents of the direct  $\text{CaO}$  and  $\text{MgO}$  determinations and those from the  $\text{K}_2\text{O}$  determinations later reported, there is still a considerable difference unaccounted for. This might probably be assigned almost entirely to sodium sulfate derived from sodium chloride in the rain waters. It was, therefore, deemed advisable to establish the occurrence of both chlorides and phosphates.

Attributing the rather heavy occurrence of chlorine as being ". . . principally furnished by the sea, fine spray of salt water being carried long distances by high winds," Lawes, Gilbert and Warrington (4) state: "In a small extent chlorides may also be furnished by combustion of fuel." In a later publication, (5) the same authors account for the excess of chlorine brought down during the winter months by stating: "The large excess of chlorides found in winter rain is probably due in great measure to the chlorides volatilized during the combustion of fuel; the excess in question is too uniform to be dependent chiefly on the action of strong winds blowing from the sea; it is also remarked in calm months as well as in stormy weather. Exceptionally high results are, however, probably due to storms."

One-liter samples of the two collections from the Knoxville stations were therefore concentrated in duplicate for the determination of chlorides and also phosphates precipitated during the first quarter of 1922. The analyses demonstrated the merest trace of phosphates in each case. However, the



TABLE 5  
Sulfates in rainfall as compared to determined total possible sulfates, when all dissolved bases present were converted to sulfate combinations, in terms of grams of  $\text{BaSO}_4$  per liter

YEAR	QUARTER	AS TO ANALYSES	KNOWVILLE UNIVERSITY FARM	KNOWVILLE WEATHER BUREAU	ODDEN FARM 7 MILES FROM KNOWVILLE	COPPER HILL OBSERV- ATORY	NEAR COPPER HILL	MCGHEE OBSERVATORY	LOUDON OBSERVATORY	CROSSVILLE FARM	MIDDLE TENNESSEE STATION FARM	WEST TENNESSEE STATION FARM
			gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.
1919	2nd	Present	0.0220	0.0600	0.0136	0.0428	0.0176	0.0188	0.0120	0.0150	0.0106	0.0376
		Possible	0.0584	0.0822	0.0220	0.0800	0.0372	0.0490	0.0620	0.1990	0.0518	0.0850
1919	3rd	Present	0.0240	0.0352	0.0112	0.0884	0.0192	0.0164	0.0078	0.0036	0.0108	0.0486
		Possible	0.0422	0.0532	0.0196	0.1024	0.0418	0.1698	0.0418	0.0660	0.0350	0.1056
1919	4th	Present	0.0272	0.3496*	0.0082	.....	0.0358	.....	.....	0.0058	0.0142	0.0230
		Possible	0.0460	1.1922*	0.0600	.....	0.0400	.....	.....	0.0356	0.1502	0.0690
1920	1st	Present	0.0360	0.0724	0.0060	.....	0.0188	0.0190	0.0146	0.0102	0.0188	0.0526
		Possible	0.0706	0.0834	0.0112	.....	0.0232	0.0628	0.0346	0.0278	0.0342	0.0788
Average		Present	0.0273	0.1293	0.0098	0.0656	0.0229	0.0181	0.0115	0.0087	0.0136	0.0405
		Possible	0.0543	0.3528	0.0282	0.0912	0.0355	0.0939	0.0461	0.0821	0.0678	0.0846

\* Melted ice.

amounts of chlorine proved to be appreciable. For the first quarterly composite of 1922, 1.45 cc. of 0.1  $N$   $AgNO_3$  was required for titration of 1 liter of concentrate of the University Farm rain water and 2.95 cc. for that from the city. If these results were taken as representative the average annual precipitation of 51 inches, would wash down 98 pounds of sodium chloride per acre at the University Farm, as compared with 199 lbs. for the city. In corresponding chlorine determinations upon the samples collected during July, August and September 0.40 cc. of 0.1  $N$   $AgNO_3$  for the University Farm and 0.675 cc. of city bureau, respectively, was required for titration. On the basis of 51-inch annual rainfall these determinations would give sodium chloride precipitations of 27 pounds and 45.6 pounds for the University Farm and city, respectively. Assuming the average of the first and third quarters to be representative of the averages of the four quarters there would be an annual precipitation of 62.5 pounds of sodium chloride at the farm and 122.3 pounds in the city. These figures represent about the same proportion that was found for the average soluble sulfate sulfur precipitations at the two points. It is quite apparent from these results that the evolution of chlorides is closely related to the evolution of soot and ash. The chlorine determinations were made for only two quarters and may not be representative of the average composition for the annual period. While not conclusive in showing the absolute amounts of chlorine brought down, the results serve as intended in confirming the assumption of the occurrence of sodium as sodium chloride and being complementary to those of the bases in accounting for the differences between soluble sulfates and total possible sulfates.

#### POTASSIUM AND SODIUM IN RAINFALL

The direct calcium and magnesium determinations of table 4 were calculated to their respective  $BaSO_4$  equivalents, in order to determine by difference what proportion of the direct sulfate determinations could be assigned hypothetically to the sulfates of these two alkali-earth bases. Only the local collections were so analyzed, since the quantity of each transported collection was limited. Table 4 shows that the difference between the  $BaSO_4$  determination and the corresponding sum of the  $BaSO_4$  equivalents varied from 4.5 mg. to 148 mg. The aggregate of calcium and magnesium fails to account for the amount of  $SO_4$  found in every instance. The results show a considerable range from a maximum of 86.7 per cent to a minimum 24.8 per cent. As an average of 13 alkaline rain waters, 42.7 per cent of determined sulfate radical was accounted for by assuming that all calcium and magnesium occurred as sulfates leaving 57.3 per cent to be accounted for by other bases. The meagerness of ammonium sulfate in the rain water was determined by analysis. The soluble salts of iron, aluminum and manganese were also present only in very minute quantities.

Since the data of table 4 indicated that the differences between the determined sulfates and the calculated sulfates may be assigned jointly to sodium

and potassium, direct determinations of potassium were made upon the first two quarterly samples collected at the University Farm and at the Knoxville Observatory. Total sulfates were determined as before and the calcium, magnesium and potassium occurrences were calculated to their respective  $\text{BaSO}_4$  equivalents, as given in table 6. Potassium salts were also found in a sample from Copperhill, but this collection was not of sufficient volume to permit of the complete analytical procedure which was carried out upon the other four rain waters. The occurrence of potassium was more uniform than either that of calcium or magnesium. The total sulfate content of the Weather Bureau sample of the first quarter was found to be nearly three times that of the University Farm collection. However, the proportions of potassium in the two samples were found to be in close agreement. In the second quarter the total potassium content of the city precipitation was considerably greater than that of the farm rain water, but the proportions of potassium were not widely divergent. On the basis of the half-year average of potassium content, the average annual precipitation of soluble potassium salts for eight years has amounted to 9.2 pounds of  $\text{K}_2\text{SO}_4$  per acre. A similar calculation from the 7-year period data for the Weather Observatory gives an annual  $\text{K}_2\text{SO}_4$  precipitation of 14.3 pounds per acre.

Thus, for each pound of soluble sulfur washed down upon an acre surface, approximately .18 pound of  $\text{K}_2\text{SO}_4$  was precipitated at the University Farm, while a corresponding proportion of 0.15 pound of  $\text{K}_2\text{SO}_4$  was found at the observatory in the city. After allowing for hypothetical sulfates from the direct determinations of calcium, magnesium and potassium, an average difference of approximately one-third may be attributed to sodium, since the possibility of lithium occurrences may be considered as negligible.

#### AMOUNT OF SOOT IN RAIN WATER AT KNOXVILLE

The foregoing results demonstrate a close parallel between the amounts of sulfur found in the rain waters and the commercial and domestic coal consumption, that is, a relation between the occurrence of soluble sulfates and the amounts of soot and ash evolved from the combustion of bituminous coal. It was thought desirable to ascertain something relative to the amount and composition of the soot found in the rain water. This may not necessarily represent all of the deposited soot. But discharged solids are a source, if not almost the entire source, of the dissolved sulfates and bases of rain water. The determinations of the absolute amounts of soot which are washed down during each season would involve a special study, and the use of specially constructed equipment. It may be well however to determine what amount of soot may be expected in rain waters before considering its composition. The amounts of soot present in the rain water collections from the University Farm and in the city were determined for the second quarter of 1922. In a 12.5-liter sample from the University Farm 0.1830 gm. of soot was found, equivalent to 0.0146 gm. per liter, or 168.6 pounds per acre in a precipitation

TABLE 6  
*Sulfates accounted for in first 6 months of 1922 rainfall by direct determinations of calcium, magnesium and potassium and assignment by difference to sodium and other bases*

COLLECTION PERIOD	WHERE COLLECTED	BaSO <sub>4</sub> EQUIVALENT OF DETERMINED Ca PER LITER		BaSO <sub>4</sub> EQUIVALENT OF DETERMINED Mg PER LITER		BaSO <sub>4</sub> EQUIVALENT OF DETERMINED K <sub>2</sub> O PER LITER		BaSO <sub>4</sub> EQUIVALENT ACCOUNTED FOR BY CaSO <sub>4</sub> , MgSO <sub>4</sub> AND K <sub>2</sub> SO <sub>4</sub> PER LITER		BaSO <sub>4</sub> EQUIVALENT ASSIGNED BY DIFFERENCE TO Na <sub>2</sub> SO <sub>4</sub> AND OTHER BASES PER LITER	
		Gram	Per cent total SO <sub>4</sub>	Gram	Per cent total SO <sub>4</sub>	Gram	Per cent total SO <sub>4</sub>	Gram	Per cent total SO <sub>4</sub>	Gram	Per cent of total SO <sub>4</sub>
January-March.....	Knoxville W. B. University Farm	0.0914	0.0240	0.0058	6.3	0.0266	29.1	0.0564	61.6	0.0350	38.3
		0.0326	0.0046	0.0008	20.9	0.0090	27.6	0.0204	62.6	0.0122	37.4
April-June.....	Knoxville W. B. University Farm	0.0258	0.0079	0.0046	17.4	0.0069	26.7	0.0194	75.4	0.0064	24.6
		0.0167	0.0046	0.0021	12.6	0.0059	35.3	0.0126	75.4	0.0041	24.6

of 51 inches per annum. In the case of the City Weather Bureau sample, 16 liters of rain water yielded 0.3005 gm. of soot, or 0.0188 gm. per liter, equivalent to 217.2 pounds per acre in an annual rainfall of 51 inches. The quantities given represent the amounts suspended in the rainfall, rather than the total amounts which may have fallen upon an acre surface.

#### COMPOSITION OF A SAMPLE OF UNLEACHED SOOT

The amounts of soot found in the rain water collections were not sufficient for analysis of sulfates and bases which may not have been dissolved from the soot flakes during the process of precipitation or the period of storage. Total sulfates were determined both in the filtered collections and in the water containing the suspended soot; but, because of the small proportion of soot to rain water, no consistent differences could be detected. Since it was not feasible to differentiate between the total and soluble sulfates and bases contained in the small amount of soot carried by the rain water, some unleached chimney soot was obtained for analysis. It might well be stressed that the total amounts of the solid products of combustion brought to the soil surface are probably considerably in excess of the amounts indicated by the rain gauges, unless precautions be taken to insure that all solid material precipitated over the surface of the collection apparatus is retained in the water samples. At times, large quantities of dry soot may be observed falling in the vicinity of much coal consumption during dry weather. It is, of course, problematical how much of this dry deposition may be dispersed by winds. The form and surface area of the rain water gauges have considerable bearing upon the amounts of solids in the collections. Lawes, Gilbert and Warrington (4) give data relative to the rainfall collections of two large oblong gauges, 6 feet by 7 feet 3.12 inches, embracing a surface area of .001 acre, as compared with those from an ordinary 5-inch circular copper gauge. Over a period of 28 years the large gauges gave a consistently greater collection of aqueous precipitation, equivalent to an average of 9.8 per cent. The disparity is most pronounced during the winter, which is the period of greater soot deposition. The authors state: "Thus a heavy snow-fall is much better retained by the large gauge than the small; the deposits of mist, dew and hoar-frost are also distinctly greater with the large gauge." It is then quite probably true that the same holds also for the deposition of dry soot, and that our own small gauges do not record the full amounts of solid material brought down.

The bituminous coal soot used for analysis was collected from a domestic boiler flue and could not be considered as absolutely comparable with the soot discharged into the atmosphere. The material discharged from the stack would have the maximum of carbonaceous matter and the minimum of the heavier ash, while the unexpelled flue soot would probably have a larger proportion of the heavier ash. The data of table 7 may be considered only as indicative of the composition of solid material carried off from the combustion of bituminous coal and brought to the soil surface by washing of the atmos-

phere and by gravity precipitations. The reaction of the soot was determined by agitating 5 gm. with 300 cc. of distilled water and permitting 20 hours additional contact before filtration and titration with 0.05 *N* acid. The entire extract required 2.7 cc. of 0.05 *N* acid to effect neutralization against methyl orange. On the basis of the amounts of soot contained in the rain waters for the first quarter of 1922, this would mean only 0.12 lb. of water-soluble alkaline material, expressed as  $\text{CaCO}_3$ , in the case of the farm precipitation and 0.15 pound at the observatory in the city.

The acid-soluble composition was determined by hot digestion of a 25-gm. charge with aqua regia. After 8 hours' digestion and two evaporations with HCl, the residue was taken up with dilute HCl, filtered and the filtrate made to a volume of 1 liter. Aliquots of 100 cc., a 2.5-gm. charge equivalent, were then used for determinations of calcium, magnesium, potassium, iron-aluminum-manganese, and sulfates. The slightly alkaline aqueous extraction obtained in the reaction determination was utilized in the determination of

TABLE 7

*Analysis of alkaline chimney soot—*aqua regia* digestion for 8 hours aqueous digestion overnight*

SOLVENT	TOTAL $\text{BaSO}_4$ FOUND	CALCIUM AS $\text{BaSO}_4$ EQUIVA- LENT	MAGNESIUM AS $\text{BaSO}_4$ EQUIVA- LENT	POTASSIUM AS $\text{BaSO}_4$ EQUIVA- LENT	CARBONATES		$\text{Fe}_2\text{O}_3 -$ $\text{Al}_2\text{O}_3 -$ $\text{Mn}_2\text{O}_3$
					$\text{CaCO}_3$	$\text{BaSO}_4$ equivalent	
Acid.....	8.57	21.89	2.21	2.85	0.27	0.63	13.06
Water.....	1.77	11.26	0.89	1.49	....	....	0

water-soluble sulfates. Water-soluble calcium, magnesium and potassium were determined by the analysis of a filtered aqueous solution obtained by overnight contact of 10 gms. of soot in a volume of 400 cc. The filtrate and washings were made to a volume of 500 cc. and aliquots representing 1.5-gm. charges of soot were used for duplicate determinations of calcium and magnesium. Potassium was determined as  $\text{K}_2\text{PtCl}_6$  upon aliquots equivalent to 1 gm. of soot. The results of the aqueous and acid digestion analyses are given in table 7. In order to facilitate comparison with the rain water results, the soot digestion data are likewise given as  $\text{BaSO}_4$  equivalents which were used in computing the total sulfates, sulfates calculated from determinations of calcium, magnesium and potassium and for sodium by difference. It will be remembered that, in the case of the rain waters, the hypothetical sulfate totals of the directly determined elements calcium, magnesium and potassium were never sufficient to account for the totals of determined amounts of the sulfate radical. The reverse is true of the acid extract from the soot. The water-soluble  $\text{BaSO}_4$  equivalent amounted to but 1.77 per cent, while the acid oxidation-digestion gave a corresponding equivalent of 8.57 per cent. The acid-soluble calcium proved to be equivalent to 21.89 per cent in terms of  $\text{BaSO}_4$  equivalence. On the same basis, 2.21 per cent and 2.85 per cent

could be attributed to magnesium and potassium, respectively. On the other hand, the water-soluble  $\text{BaSO}_4$  equivalents of calcium, magnesium and potassium were found to be but 11.26 per cent, 0.89 per cent and 1.49 per cent, respectively. No trace of either iron or aluminum was found in the slightly alkaline aqueous extract. In connection with the alkalinity determinations which demonstrated a paucity of alkali carbonates, these results suggested the possibility of the presence of considerable amounts of the alkali-earth carbonates. However, such was proved not to be the case. A boiling digestion with 0.5 *N* acid gave a  $\text{CaCO}_3$  equivalence of only 0.27 per cent or a  $\text{BaSO}_4$  equivalence of 0.63 per cent. These analyses indicate that considerable quantities of non-carbonaceous ash, most probably siliceous, are present in the flue soot. The bases of the non-carbonate ash were tightly locked; for, in spite of the alkalinity of the dry soot, the iron pipe from which it was obtained had been attacked as though by an acid and ferrous sulfate was

TABLE 8

*Hypothetical depositions of sulfur, calcium, magnesium, potassium and  $\text{Fe}_2\text{O}_3\text{-Al}_2\text{O}_3\text{-Mn}_2\text{O}_4$  per acre from soot suspensions in 51 inches of rainfall calculated from data of tables 6 and 7*

PLACE	TOTAL $\text{BaSO}_4$	CALCIUM AS $\text{BaSO}_4$ EQUIVA- LENT	MAGNESIUM AS $\text{BaSO}_4$ EQUIVA- LENT	POTASSIUM AS $\text{BaSO}_4$ EQUIVA- LENT	CARBONATES		$\text{Fe}_2\text{O}_3 -$ $\text{Al}_2\text{O}_3 -$ $\text{Mn}_2\text{O}_4$
					$\text{CaCO}_3$	$\text{BaSO}_4$ equiva- lent	
	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>
University Farm.....	14.2 (3.0*)	36.9	3.7	4.8	0.5	1.1	22.0
City Weather Bureau.....	18.6 (3.8*)	47.5	4.8	6.2	0.6	1.4	28.4

\* Water-soluble.

indicated by taste. During periods of no heat and condensation of moisture there was apparently an oxidation of sulfur compounds and formation of acid salts most probably of iron, the  $\text{Fe}_2\text{O}_3\text{-Al}_2\text{O}_3\text{-Mn}_2\text{O}_4$  content of the soot amounting to 13.06 per cent.

#### SULFUR AND BASES BROUGHT DOWN BY DRY SOOT

As previously noted, considerable quantities of dry soot are often deposited. Analyses of dry chimney soot indicate that not all sulfurous and basic materials carried by the rainfall-soot are in forms which permit of their solution during precipitation, or while standing in the quarterly-collection containers. Applying the soot data of table 7 to the amounts of soot found in the two quarterly rain water collections, and assuming those collections to be representative of the 51-inch average annual precipitation, the pounds per acre data of table 8 are obtained. These figures indicate that the actual sulfur precipitations, and more particularly the wash-down of lime and the amphi-

teric elements, are apt to be somewhat greater than the soluble amounts found in the rain water. Furthermore, gravity precipitation of dry solids augments the amount of sulfur and bases brought to the soil surface. The large excess of basic elements over sulfate and carbonate radicals, together with absence of appreciable basicity, the mere traces of phosphates and the chlorine content, lead to the conclusion that the soot-carried bases are most probably present as silicates. The approximate ratio of 5 parts of total sulfur to 1 part of water-soluble sulfur establishes the fact that the amount of precipitated non-sulfate sulfur varies directly with the amount of the solid soot which may fall in the dry form or along with the rain water. The same is true of ferric, aluminic and manganic oxides, for we have found but traces of the salts of these three elements dissolved in the quarterly rainfall collections.

#### PRACTICAL SIGNIFICANCE

The results of this study serve to refute some of the viewpoints which have been commonly held relative to the effect of sulfur precipitations upon soils and plant growth in commercial centers, other than those where sulfur is evolved from smelter operations or chemical manufacture. It has been held that sulfur evolutions from coal produce an acid rainfall which depletes the soil of lime and other bases, thereby causing acidity. The writer has known of computations made to show the tonnage of lime required to offset the precipitation of supposed acidity throughout a state, on the assumption that the sulfur evolved from coal combustion, or rather destructive distillation was in the acid combination. The layman has likewise insisted that corrosion of gutters and other exposed metal surfaces is due to this assumed acidity of rain water. Our findings do not support such conclusions. The consistent results upon the reaction of the rain waters at different points and the excess of soluble bases over the soluble acid radicals, indicate that soils are not more depleted of bases, nor made more acid by rainfall than they would be if the precipitations were of pure water. Furthermore, in the case of the two locations, Knoxville and the University Farm, the amounts of calcium, magnesium, potassium and sulfate sulfur reach such proportions as to be considered of some possible manurial value. There is certainly just as much justification for the assumption that the rainfall components are beneficial, as that to the contrary.

#### SUMMARY

Analyses were made quarterly of the rain water collected at 10 different points in Tennessee.

The 8-year average of annual soluble-sulfate sulfur precipitations at the University Farm was 51.5 pounds per acre. The 7-year average in the heart of the city was 94.5 pounds. The 2-year average 7 miles from Knoxville was 18.6 pounds. Three-year averages at seven other points were as follows:



Copperhill, 232.4 pounds; near Copperhill, 72.2 pounds; McGhee, 24.2 pounds; Loudon, 19.3 pounds; Crossville, 12.7 pounds; Columbia, 26.2 pounds and Jackson, 59.2 pounds.

From duplicate titrations of over 300 samples it was found that every sample was alkaline to methyl orange, while slight acidity was indicated by cochineal in only two instances.

Considerable amounts of calcium and magnesium were found in all of the seven samples from the Knoxville Observatory and the six from the University Farm which were analyzed for these elements. Forty-two and seven-tenths per cent of the soluble sulfates were accounted for by those two elements, as the average from the 13 samples, 26.1 per cent by calcium and 16.6 per cent by magnesium.

Determinations were made upon the samples from all ten sampling points for the sulfate content and the sulfates that would be present if all dissolved bases were combined with the sulfate radical. As an average of 36 duplicate determinations, the sulfates found proved to be but 35.9 per cent of the possible.

Chlorine determinations and tests for phosphate were made upon two quarterly samplings from the two Knoxville collection points.

Sodium and potassium combinations were first determined jointly by difference between total sulfates and those of calcium and magnesium. Potassium was later determined directly. The totals of potassium, calcium and magnesium calculated to sulfate equivalents, established the fact that considerable sodium sulfate was present in the analyzed samples.

The potassium content of rain water was rather uniform. About 30 per cent of the sulfates found were accounted for by this element.

The amounts of suspended soot in certain rain waters were determined.

The water-soluble and acid-soluble composition of unleached soot were given. The former was, on the average, about one-half of the latter.

The relation between the deposition of sulfur, calcium, magnesium, potassium and sodium brought down by gravitation of dry soot and that washed down was considered.

It was pointed out that most of the soluble sulfates found in the rain waters have been dissolved from solid matter and that the volume of such discharged into the atmosphere is the primary factor governing the concentration of sulfates in the rain water.

As a practical application of the findings reported, it is believed that, under the conditions studied, the rainfall carrying sulfurous materials can not be assumed to be more active than pure water in producing acid soils.

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## THE SOIL SOLUTION AND ITS IMPORTANCE IN THE GROWTH OF PLANTS<sup>1</sup>

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The complex relationships of soil, climate and specificity of the nature of the cultivated plants lead to varying yearly crop yields. To be able to differentiate the yearly relationship between the soil, weather conditions and plant, the individual factors influencing the growth of the plants have to be differentiated and their importance determined.

Agriculture is always determined in arid regions by that quantity of atmospheric precipitation, which occurs during the period of plant growth and the period of soil preparation previous to the seeding. However, the quantity of precipitation during the whole vegetation period does not yet determine the quantity of the yield—the amount of precipitation is particularly important at definite periods of the growth of plants, while at other periods it is either of little use or entirely useless.

A larger or smaller quantity of rainfall increases the quantity of soil moisture and there is a corresponding change in the concentration of the soil solution; this in its turn, influences the entrance of the dissolved nutrients into the plant roots. Investigations of the importance of osmotic pressure of the soil solution upon the growth and yield of spring wheat were begun by the author in 1910 at the Bezentschuk Experiment Station, in the state of Samara. These investigations are still being continued and are devoted, not to the development and yield of wheat, but to its chemical composition and protein content.

The original experiments on various salts of the same concentration convinced us at an early date that the same concentration of the soil solution produced by various salts, and therefore of different osmotic pressure, does not act alike upon the growth of plants. At first we followed the swelling and the germination of the grain and confirmed the results of earlier workers, viz., Buffum, and Slosson at the Wyoming Experiment Station. We then began to investigate the growth of plants in pots, in extensive experiments at the Bezentschuk Experiment Station during 1910-1916, then in Petrograd during 1917-1920, and finally in Saratov since 1921.

The experiments were conducted with some local soil, a black soil (tschernozem) in Bezentschuk and Saratov and a clay soil in Petrograd, in ordinary Wagner pots, 20 cm. in diameter and 20 cm. high. To 5 kgm. of absolutely dry soil, all the minerals were usually added which would favor the maximum

<sup>1</sup> This is merely a summary of extensive investigations on osmosis. The detailed studies will be published later.

crop-yield. The osmotic pressure of the soil solution of a normal pot containing the soil, nutrients and 60 per cent of the moisture-holding capacity renewed daily, was taken as a unit of comparison; this was increased in other vessels by the addition of common salts, NaCl, Na<sub>2</sub>SO<sub>4</sub>, MgCl<sub>2</sub>, NaNO<sub>3</sub>, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, NH<sub>4</sub>NO<sub>3</sub>, etc., in such quantities as to increase, when in water solution, the osmotic pressure by 1, 2, 3 atmospheres, etc. These salts were usually added in full amounts when filling the pots with the soil, and sometimes the addition of salts was extended during the whole period of growth of the wheat. At first, wheat seed, *Belaturka* (*Tr. V. hordeiforme*) was taken from a general lot of seed, but later a purer strain was selected at the corresponding Experiment Stations.

Growth, yield, hygroscopic water, total nitrogen and protein and later the hardness and softness of the grain when cut were determined.

#### INFLUENCE OF OSMOTIC PRESSURE ON THE GROWTH OF WHEAT

An increase in osmotic pressure of the soil solution arrested to a marked degree the sprouting of the seed and weakened it considerably. It seems that the more concentrated solutions enter the seed much more slowly and germination begins later. All the following phases of growth were also arrested; after the period of blossoming was over and ears were formed, the period of ripening was considerably hastened and the total period of vegetative growth for plants upon soil with an increased osmotic pressure was usually markedly less than in normal soils, usually by 6-7 days. In the presence of nitrates the plants behaved differently, since the nitrates, as is well known, extend appreciably the vegetative period of the growth of wheat. Although an increased osmotic pressure of the soil solution arrested the growth of the plants, it is important to note that, with a certain optimum osmotic pressure, the development of the plants reached its maximum. The growth of wheat plants obtained in 1914 and 1915 is given in table 1.

Table 2 gives the influence of various osmotic pressures upon the yield of tops. The data are given for the average of the salts grouped according to their osmotic pressures.

These data definitely established the fact that, with an increase in osmotic pressure of the soil solution, there is an increase in the yield of tops until a certain optimum is reached; on further increasing the osmotic concentration of the soil solution above certain limits there is a decrease in yield. The yield of wheat grain per vessel in relation to the osmotic pressure is given in table 3, which agrees closely with table 2. The data are averages of the results obtained for the same period for all the salts.

From numerous experiments with various salts, it can be concluded that an increase in osmotic pressure of the soil solution up to a certain limit, caused by introducing nutritive salts into the soil, will bring about a greater activity of the plants; under certain conditions there will be an increase in size, yield

of tops and yield of grain of spring wheat. Further increase of osmotic pressure decreases the yield of tops and grain.

As stated above, the wheat grain was analyzed, with special emphasis laid upon the nitrogen content as characterizing the amount of protein in the grain, and, therefore, the quality of the grain itself. A great deal of information has been obtained on this subject, which definitely establishes the character of the influence of an increased osmotic pressure of the soil solution upon the nitrogen and protein content of wheat grain. Since all the data on the nitrogen content of wheat grain grown under different osmotic pressures of the soil solution cannot be presented here, we can only give the averages for different salts and for different years, the data for most salts being for the years 1912 and 1915.

TABLE 1  
*The average height of wheat in pots for two years*

YEAR	NORMAL	1 ATM.	2 ATM.	3 ATM.	5 ATM.	7 ATM.
	cm.	cm.	cm.	cm.	cm.	cm.
1914	113.2	126.5	116.6	115.5	103.6	84.0
1915	120.2	132.8	125.2	111.6	83.9	72.6

TABLE 2  
*Average weight of tops per pot in 1914-15*

NORMAL CULTURE	1 ATM.	2 ATM.	3 ATM.	5 ATM.	7 ATM.
gm.	gm.	gm.	gm.	gm.	gm.
54	56.9	57.6	52.3	35.8	18.2

TABLE 3  
*Average yield of grain per vessel*

NORMAL	1 ATM.	2 ATM.	3 ATM.	5 ATM.	7 ATM.
gm.	gm.	gm.	gm.	gm.	gm.
20.2	22.2	22.3	20.3	13.2	6.3

Table 4 definitely establishes the fact of increase of total nitrogen and protein nitrogen in particular in the grain of wheat by an increase in the osmotic pressure of the soil solution.

The results of these determinations clearly indicate that the increased content of nitrogen and protein in the wheat grain of southeastern Russia and the arid parts of the United States is doubtlessly connected with the increase in osmotic concentration in the soils of these sections because of the comparatively small amount of rainfall in those sections during the growth of wheat. Table 5 shows the nitrogen content of the grain calculated from tables 3 and 4 and expressed in terms of the normal pot as 100. This indicates that an increase in osmotic pressure caused an increase of the

total nitrogenous substances in the grain of wheat. Even where the total yield of grain was comparatively small, its nitrogen content was such that the total nitrogen yield was greater than in normal pots. As in all the previous cases, the increase to 2 atmospheres osmotic pressure was most favorable; not only was the growth of wheat best in this case, but also the greatest nitrogen yield was obtained per field plot or pot.

It was noted that the grain of the same wheat in the pot studies varied in quality as different quantities of salts were added. In the pots with a normal soil solution there was a large quantity of soft grains together with purely hard grains; in the pots with an increased osmotic pressure, the wheat grains were always all hard. Table 6 shows results obtained with sodium sulfate in 1915. It is thus clear that the quantity of hard grain is increased with an increase in osmotic pressure and *vice versa*, with a comparatively low osmotic pressure of the soil solution, the grain of the same wheat is almost fully soft.

This information allows us to believe that, in vegetative experiments, the same variety of wheat can be made to produce both hard and soft grain.

TABLE 4  
*Nitrogen content of the grain, Belaturka wheat*

	NORMAL POT	1 ATM.	2 ATM.	3 ATM.	5 ATM.
	per cent	per cent	per cent	per cent	per cent
Total nitrogen.....	1.758	2.076	2.342	2.520	2.773
Protein nitrogen.....	1.647	2.014	2.247	2.518	.....

TABLE 5  
*Relative nitrogen content of grain*

NORMAL POT	1 ATM.	2 ATM.	3 ATM.	5 ATM.
100	129.8	146.3	143.0	109.2

The experiments of 1915 were continued the following years including 1922, and the information obtained in this period of time fully confirms this conclusion. We also noted that by introducing various salts into the soil, we could influence still further the character of softness and hardness of the grain. It was found that nitrates bring about greater increase in hardness than sulfates and that, in addition to the influence of the osmotic pressure of the soil solution, one has to note also the nature of its constituent salts.

Numerous data which have accumulated during the last 12 years indicate rather clearly that the osmotic pressure of the soil solution influences the transpiration coefficient of the plants studied. Table 7 shows the relationships observed in the experiments of 1914 and 1915 as averages for all salts used. Increasing the osmotic pressure of the soil solution, decreased the quantity of water required to form 1 gm. of crop yield. The plants, therefore, used the soil moisture more economically with an increase in the osmotic pressure of the soil solution.

In the last few years, these investigations have been extended to include other plants. The chief object has been to find out whether an increase in osmotic pressure of the soil solution has an influence upon the formation of other substances, such as fats, sugars, aromatic bodies, etc. These experi-

TABLE 6  
*Hardness and softness of wheat grain at different osmotic pressures*

OSMOTIC PRESSURE	SOFT GRAIN	HARD-SOFT GRAIN	COMPLETELY HARD GRAIN
<i>atm.</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
0.5	27.8	70.0	2.2
3.0	3.6	83.0	13.4
5.0	1.7	48.0	50.3
7.0	0	0	100.0

TABLE 7  
*Transpiration coefficients with different osmotic pressure*

NORMAL CULTURE	1 ATM.	2 ATM.	3 ATM.	4 ATM.
365.6	348.6	333.3	329.9	314.8

ments have not given as yet definite results, since they could not be carried out as completely as necessary, but the results obtained suggest several hypotheses. The work is being carried out with oil-containing plants (flax and mustard) and aromatic plants (mints) and is planned for sugar beets.





## ON THE QUESTION OF OBTAINING THE SOIL SOLUTION

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The moisture and nutrition factors of southeastern Russia play an important part both in the life of plants as well as in the soil-forming processes. The Department of Soils, in studying the questions of the life of the soil, has, therefore, taken up the study of the dynamics of the soil solution as one of the principal factors of plant growth and for the purpose of throwing light upon the genesis of local soils.

In carrying out the work outlined, the usual methods of obtaining the soil solution were of little value. The most common method of obtaining the soil solution, that of Morgan, consisting in mixing the soil with petroleum oil, pressing and centrifuging, can give, for the dry soils of the Southeast, only a small quantity of soil solution, with which it is very difficult to operate in physico-chemical analysis. By means of the method of L. I. Briggs and I. W. Mehane based on the action of centrifugal force, only a small quantity of the soil solution is obtained even with a comparatively high moisture-content of the soil. The method of Ischtscherekov, consisting in pressing out the soil solution by means of alcohol, has the same disadvantages as the previous methods. Other methods could not be utilized in the laboratory due to technical difficulties through which the country was passing.

These considerations led to the necessity of developing a special method for obtaining the soil solution in a comparatively large quantity more quickly. An apparatus was constructed for this purpose which is based upon the evacuation of the atmosphere within a hollow cylinder, placed within a definite volume of soil. The pressure of the outer atmosphere displaces the water from the soil into this cylinder, as soon as sufficient vacuum has been produced. To obtain sufficient soil solution with a moisture-content of less than 50 per cent saturation and to counteract the molecular forces in the soil, which tend to hold small quantities of moisture, the soil has been pressed by means of a common press in addition to the air evacuation. The apparatus is shown in figure 1. It consists of combination of a Karting suction pump *A* of which the high-vacuum cylinder *B* is a part, press *C*, hollow plates *D*, metallic cup *F*, potassium tube *E*, and vessel *G*.

Cylinder *B* consists of a metallic netting of 8-mm. mesh fused where wires cross to give added strength.

A stopper with a tight fitting glass tube is fitted into the upper part of

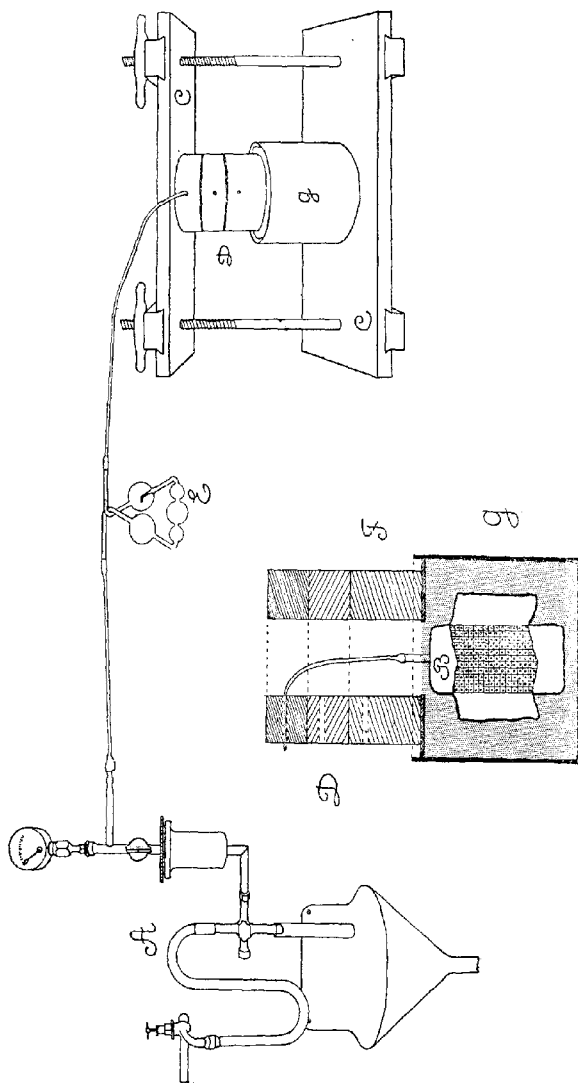


FIG. 1. DIAGRAM OF APPARATUS FOR EXTRACTION OF SOIL SOLUTION BY ATMOSPHERIC PRESSURE.

White flays represent batiste, turned back

the cylinder. The net is then paraffined, which reduces the mesh to 3-5 mm. in diameter. It is then tightly covered with batiste to exclude soil particles. The volume of the cylinder varies from 10 to 150 cc., depending on the quantity of soil from which the soil solution is to be extracted. The cylinder is connected with the suction pump by means of rubber tubing through the potassium tube E which serves as an indicator of the action of the pump and the whole apparatus.

The compacted soil adhering to the outside of the cylinder prevents the return of moisture to the soil.<sup>1</sup>

In adapting the press to the apparatus, a metallic plate *F* is placed upon the surface of the soil in the vessel *G*; the plate is then connected by a system of cylindrical plates *D* with the upper cover of the press. The metallic plate and the vessel are covered inside with paraffin.

In obtaining the soil solution, it is important to see that the rubber tubes connecting the parts of the apparatus fit well and also that the soil adheres well to the cylinder so that a vacuum is formed when the pump is working. The last factor is particularly important when the moisture-content of the soil is low. The soil is, therefore, compressed slightly by means of the press preliminary to the action of the suction pump. The pump is then started and as the volume of the soil in the vessel is diminished, pressure is gradually applied to the soil. The first drops appearing in the potassium tube indicate that the cylinder is filled with the soil solution which is then taken out by means of a siphon placed within the cylinder. The soil solution thus obtained is usually slightly turbid and opalescent, particularly the first portions. It is, therefore, filtered through filter paper immediately. It takes 4 to 30 minutes to extract the soil solution by the above method, depending on the moisture-content of the soil. With a moisture-content greater than 25 per cent of the weight of absolutely dry soil, 200 cc. of soil solution is obtained in 10-15 minutes.

Extracts of the cultivated portion of a dark-brown soil were made by this method. Until the present, soil solutions were obtained from this soil by the use of suction and pressure with a moisture-content of 17 per cent of the absolutely dry weight. To characterize the chemical and physical properties of the soil solution obtained by this method we may cite the data obtained from experiment 17. The soil was taken from a portion of the fallow land of the experimental field of the station; the moisture was brought to 38.7 per cent of the absolutely dry soil, corresponding to 90 per cent of the total moisture-holding capacity, and kept at that moisture for 13 days. The soil solution was then extracted. To determine the uniformity of the soil solution, it was taken out in 15 separate 60-cc. portions. A total of 894 cc. of soil solution was obtained from a total of 2250 cc. present in all the soil.

<sup>1</sup> This apparatus has been modified by substituting for the metallic net a copper cylinder perforated with 3-4 mm. holes. This cylinder is not paraffined but is covered with batiste. Instead of using a rubber stopper and glass tube, connection with the potassium tube *E* is obtained by extending the upper part of the cylinder into a tube of the same copper over which the rubber tubing is fitted.

After the extraction was completed, soil samples were taken from the different parts of the vessel, for moisture determinations which latter varied between 6.9 and 17.4 per cent of the absolutely dry weight of soil. The results of the analysis of the consecutively extracted portions of the soil solution are given in the tables 1 and 2.

The figures obtained definitely demonstrate the constancy of the concentration of the soil solution. The determination of phosphoric acid in the soil solution did not present any difficulties due to the high concentration of the latter in comparison with a water extract of soils. The difference in the concentration of the solution obtained by the method described here in comparison with a water extract is well illustrated in the data from experiment No. 4. By determining the nitrates in the soil sampled the February 10, 1922, by

TABLE 1  
*Osmotic pressure and dry matter in the soil solution*

NUMBER OF PORTIONS OF THE SOIL SOLUTION	OSMOTIC PRESSURE	DRY MATTER ON THE BASIS OF 1 KGM. OF ABSOLUTELY DRY SOIL
	<i>atmospheres</i>	<i>gm.</i>
1 and 2	0.54	0.55
6	0.49	0.50
10	0.51	0.52
12	0.51	0.52
15	0.50	....

TABLE 2  
*Nitrates, phosphates and organic matter in the soil solution*

NUMBER OF PORTIONS OF THE SOIL SOLUTION	NITRATES PER 1 KGM. OF DRY SOIL	P <sub>2</sub> O <sub>5</sub> PER 1 KGM. OF DRY SOIL	ORGANIC MATTER PER 1 KGM. OF DRY SOIL
	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>
1, 2 and 3	11.78	0.71	16.80
5, 6 and 7	11.69	0.73	15.68
10, 11 and 12	11.87	0.73	15.98

the method of extraction with water, 2.1 mgm. were found per kilogram of absolutely dry soil. By changing the moisture-content of the soil to 80 per cent of the total moisture-holding capacity, mixing the soil with the water for 3 minutes, as in the case of preparing the water extract, then extracting the soil solution by the above method which consumed 2 minutes, 30 mgm. NO<sub>3</sub> were found per kilogram of absolutely dry soil. On repeating the experiment with soil samples taken April 11, analogous results were obtained.

Since it was possible now to obtain the soil solution in comparatively large quantities, experiments were carried out on the growth of plants in the solution by the method of water cultures. Wheat, oats, barley, peas, etc. were grown in the green house, to full maturity on a soil solution taken from a field soil with the natural moisture-content. By the use of this method, it is now possible

to determine the osmotic pressure and chemistry of the soil solution in the soil of pot cultures in the different periods of growth of cultivated plants.

It should be noted that the work outlined on the method of obtaining the soil solution is just begun. The data obtained, however, allow us to draw the following conclusions:

1. This method permits extraction from varying quantities of soil, within the limits of 100 gm. and 6-7 kgm.
2. There is more of the soil solution obtained by this method than by the other methods.
3. Comparatively little time is consumed for obtaining the soil solution by the method described.
4. The soil solution obtained is of constant concentration in the consecutive portions.
5. By changing the ratio of water to soil more concentrated solutions are obtained for chemical analysis than by water extracts of the soil.



# MICROBIOLOGICAL ANALYSIS OF SOILS AS AN INDEX OF SOIL FERTILITY: V. METHODS FOR THE STUDY OF NITRIFICATION<sup>1</sup>

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## INTRODUCTORY

In the study of any soil bacteriological phenomenon, we must differentiate between the activities of the microorganisms themselves and the influence of the chemical condition of the soil upon them. From a practical point of view, however, particularly when a correlation is sought between the bacteriological function and crop productivity, a fine differentiation between the bacteriological and chemical soil condition could hardly be expected and may be entirely unnecessary.

When equal amounts of soil are introduced into sterile media of the proper composition and the transformations taking place are studied, we may be able to differentiate between the relative abundance of specific bacteria and perhaps also point out differences in fertility. The information thus obtained will not, however, tell the whole story: a physiological group of microorganisms may be represented in two soils by equal numbers, which may be of different efficiency. If conditions are favorable (selective) for the activities of one particular group of microorganisms and there is a long incubation period, the finer differences between activities of this particular group in the various soils will tend to be obliterated. This is true both of the original Remy method (38) and the modification suggested by Löhnis (31, 32), whereby soil extract is used in the medium.

The use of fresh soil, to which one ingredient is added and the change of which is studied, would seem to present a more natural condition than the solution method. But here again, we must carefully consider the transformation that we are studying. As pointed out by Meyerhof (35), nitrification is at an optimum when the reaction is distinctly alkaline; with an increase in acidity, nitrification rapidly decreases. When we compare, therefore, the

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nitrifying capacity of two soils, one more acid than the other, or one unlimed and the other limed, the former is bound to give a lower nitrifying capacity using ammonium sulfate or a high grade organic nitrogenous material even though it may be a more fertile soil. When we compare the nitrifying capacity of two soils by the common method of adding ammonium sulfate (much in excess of what we would add to field soils) to 100 gm. of soil and determining the nitrates formed after incubation, we actually measure not the nitrifying capacities of the two soils but merely, in a roundabout way, the initial reaction, buffer content, and presence of neutralizing substances of the soil. The latter could be done, within a shorter period of time and more accurately by chemical methods. Dried blood has found favor with some bacteriologists as a substratum for the study of nitrification; this is, no doubt, due to the fact that the ammonia rapidly produced from dried blood in acid soils tends to make the reaction of the soil less acid and, ipso facto, make conditions more favorable for an abundant accumulation of nitrates.

In studying a bacteriological function, such as nitrification, for the differentiation of soil fertility we must, therefore, make certain that it is only the function in question that is studied, including the chemical conditions of the soil (such as stimulants, etc.) influencing it. When we use it, however, as an index of a certain chemical condition of the soil, such as soil reaction, abundance of lime or available phosphates, we must definitely differentiate between this information and the general consideration of soil fertility.

#### HISTORICAL

For the study of nitrification in soil, Remy (38) used at first two solutions, one with ammonium salt as a base and one with nitrite. Löhnis (31, 32) limited himself to one solution containing only 0.1 per cent ammonium sulfate; 5 gm. of soil or soil suspension were inoculated into 50 cc. of medium and incubated 30 days at 20°C. and then the nitrates were determined quantitatively. Barthel (5) suggested aeration of the flasks for 20 days at 20°C.

The use of liquid media for the study of nitrification has met with a great deal of criticism and has been found by some investigators to be much less favorable than the use of soils, since nitrifying bacteria are more sensitive to organic substances in solution than in soil. Stevens and Withers (41, 42) have shown that soil organisms nitrify less in liquid than in soil, while bacteria from sewage purification act better in liquid media. No relation was found between bacterial activities in soil and in a suspension of the same soil. Tests in solution were found inadequate for indicating the nitrifying power of a soil, and soil itself was recommended as a medium for the study of nitrification. Similar observations as to the study of nitrification in liquid media were made by Koch and Petit (27), Koch (26), Fischer (10), Heinze (19), Lemmermann and associates (28), and Vogel (45). Kellerman and Allen (22), using the soil method, obtained not only a close correlation between nitrification and soil productivity, but even a definite differentiation between soil samples taken at different depths. By the use of the solution method, however, no differentiation was obtained between a good and a poor soil and, in some cases, samples taken from the lower depths allowed as much nitrate accumulation as the surface samples. Nitrification of organic materials cannot be studied in liquid media due to the toxic effect of the soluble organic compounds and the ammonia formed. Kellerman and Robinson (23) further state definitely that nitrifying bacteria do not act normally in test solutions.

Löhnis (33), Löhnis and Green (34) and Green (17) discussed the subject in detail in later contributions and found that, for the study of nitrification, solution methods are quite as valuable as methods in which the natural soil is used as a medium. They very properly pointed out that a low lime content may act as a limiting factor to nitrification in soil tests, when ammonium sulfate is used, although not in the field, so that the method of study of nitrification in soil may be merely an index of lime content rather than of nitrifying efficiency. In the field, where only relatively minute quantities of ammonia are to be nitrified, the low natural lime content need not enter as a limiting factor.

A correlation between soil type and nitrification by the solution and soil method was found by Fischer (10), but the results obtained by the latter method were more pronounced. A definite correlation was found by Wohltmann, Fischer and Schneider (51) between soil fertilization and nitrification (lime was found to have a particularly favorable effect on nitrification of ammonium sulfate) in the soil as well as in solution, but the differences found by the latter method disappear on prolonged incubation.

The solution method for the study of nitrification gives, however, information that could not possibly be obtained by the soil method alone, such as the distribution of the organisms noted by Allen and Bonazzi (2), activities of the organisms not in their natural substrata, as well as in very adsorptive moor soils mentioned by Ritter (39). If the results obtained by the solution method do not give a direct absolute comparison with soil productivity, the relative comparison may be of great importance in supplementing the information obtained by the soil method.

The soil method, as commonly carried out, consists of adding a certain amount of an inorganic salt of ammonium, usually the sulfate, 0.1 to 1.0 per cent, or organic nitrogenous material, usually dried blood, at the rate of 0.5 to 2.0 per cent to 1-100 gm. of soil. The soil is kept at optimum moisture for 10, 20 or 30 days, at a temperature of 25 to 28°C. The nitrates formed are determined either by the phenol-di-sulfonic or by reduction methods.

Temple (43, 44) found that various nitrogenous organic substances and ammonium salts of organic acids nitrified in the soil faster than the ammonium sulfate and chloride. This was not due to the nature of the nitrifying organisms in the soil, since pure cultures of nitrifying organisms obtained from a number of sources produced the same result. When  $\text{CaCO}_3$  was added to the soil, ammonium sulfate nitrified as well as any of the other substances. These soils (of the Cecil group) were all acid; the soil organisms decomposed the substances of organic origin in a way that more ammonia than acid was produced thus correcting the acidity and bringing about a condition favorable for the growth of the nitrifying organisms. With ammonium sulfate and chloride, the soils became even more acid due to the liberation of the anion and transformation of cation into nitric acid. When lime was added to the soil, the relation was reversed.

Fred and Graul (12) also found that, in acid soils, organic nitrogen nitrifies much quicker than ammonium sulfate, regardless of the source of nitrifying bacteria. In neutral or alkaline soils the reverse takes place. Barthel (6) suggested that the poor nitrification of ammonium sulfate in acid soils is due to the fact that, as soon as nitrification begins, nitric and sulfuric acid accumulate in the soil and reduce the hydrogen-ion concentration of the soil to a degree, which checks the further development of the nitrifying bacteria. But, when lime is added, the acid is neutralized, and no increase in the hydrogen-ion concentration of the soil will take place.

The lack of nitrification in a certain soil need not necessarily indicate the absence of nitrifying organisms, but merely an unfavorable chemical condition of the soil, as shown by Arnd (4) in his study on nitrification in peat soils. Raw, unlimed peat soils, acid in nature, are unable to nitrify the ammonia formed in the soil from the decomposition of organic matter or when added directly to the soil. When enough basic material ( $\text{CaCO}_3$ ) is added to neutralize the acid, active nitrification takes place. Low peat soils, of a neutral reaction, need no liming for nitrification. An increase in lime much above the neutral point may result in lower nitrate accumulation.

The use of inorganic salts of ammonia and organic materials has not found equal favor in the hands of the various investigators. Some have favored one; some, the other, as giving a true picture of the nitrifying capacity of the soil. Some have used both methods, without trying to explain the difference in results obtained by the two methods. The peculiar influence of lime on nitrification, both on the oxidation of inorganic and organic materials, has been observed by various workers. No attempt has usually been made (except by Barthel (6) and a few others to correlate this with the original and final soil reaction as well as the influence of ammonia produced from the decomposition of the organic material, under alkaline conditions, on the action of the nitrifying bacteria. Beckwith and associates (7), for example, found active ammonification taking place in six Oregon soils, but in two soils, less nitrate was found after four weeks incubation, both with dried blood and ammonium sulfate, than in the original soil itself. In one case the further addition of lime failed to induce the nitrification of these materials, although the nitrifying bacteria were present.

One is almost certain to find, by going over the literature on nitrification, that ammonium sulfate is not nitrified or only to a small extent in acid soils, particularly soils that are poorly buffered; but it is very vigorously nitrified in neutral and alkaline soils, particularly rich in organic matter. Lipman and Burgess (30) found the latter to be true for California soils, while Temple (44) and others found the former to be true for acid soils.

Dried-blood and high-grade tankage, as well as other high-grade nitrogenous materials will nitrify well in not too acid soils, particularly in well buffered soils, but they will not nitrify readily in alkaline soils, or in soils poorly buffered (with low humus content). Low grade nitrogen materials will nitrify better in soils with a low buffer content, due to the fact that the relatively smaller ammonia formation will not tend to injure the activity of the nitrifying bacteria, while these materials will offer a greater buffering agent to the accumulation of nitric acid. High grade nitrogenous materials, particularly in amounts used for laboratory tests, allow such a rapid and intense accumulation of ammonia that the nitrifying bacteria are injured in their activities. The injurious effect of ammonia on nitrification has been long recognized but, while Winogradsky (50) believed that ammonium sulfate was also toxic, Meyerhof (35) demonstrated that it is free ammonia and not the ammonium salt which injures the nitrate-forming bacteria and that the salt is uninjurious even in solution, in the presence of buffering substances like phosphates. Under alkaline conditions, particularly in the presence of sodium carbonate or bicarbonate, the ammonium salt will interact with the carbonate giving free ammonia. In soils with a low humus content, particularly of a neutral or alkaline reaction, there are not enough acid radicals or buffering materials to combine with the ammonia formed abundantly from the high grade nitrogenous material used for the test, and the latter becomes injurious.

This explains also the observations made that small amounts of  $K_2CO_3$  stimulate nitrification, while larger applications of the salt progressively diminish the rate of nitrification. It explains also the fact that  $CaCO_3$  may have a retarding effect on nitrification, particularly of blood-meal, as found by Beckwith and associates (7), or a favorable effect on the nitrification not only of ammonium salts, but organic nitrogen compounds, as shown by Fred and Graul (12) who worked with acid soils.

Kelley (24, 25) found that nitrate formation from dried blood, bone meal, and ammonium sulfate during four weeks incubation varied enormously when different concentrations were employed. With 1 per cent of dried blood, nitrification was feeble or absent in certain soils in which 1 per cent of bone meal and 0.2 to 0.3 per cent of ammonium sulfate underwent active nitrification. When low concentrations of dried blood were employed, such as are used in the field, active nitrification took place in every case. High concentrations of bone meal with a nitrogen content corresponding to that furnished by 1 per cent of dried blood were also toxic to nitrification. It is interesting to note that 0.05 per cent sodium carbonate was distinctly toxic to the nitrification of 1 per cent of dried blood, while as high a concentration as 0.4 per cent sodium carbonate produced no effects on the nitrification of 0.1 per cent dried blood. This bears out the previous discussion. That sodium carbonate is far

more toxic to nitrification of a high concentration of dried blood than sodium sulfate has been pointed out by Lipman (29); 0.1 per cent sodium carbonate was toxic to 0.15 per cent of ammonium sulfate, and markedly stimulating to 0.0625 per cent. Kelley (25), therefore, concluded that nitrification studies, in which high concentrations of nitrogenous materials are added and nitrates determined at a fixed interval of time, are likely to be more misleading than informing. Kelley recognized quite correctly that the intermediate products formed in the process of nitrification may, directly or indirectly, exert much influence upon the oxidation of ammonia. He states elsewhere (24) that  $\text{CaCO}_3$  exerts almost no effect on the nitrification of dried blood in the soil. This is of course explained by the facts that the injurious influence of high concentration of dried blood is due to an excess of ammonia formation, particularly in poorly buffered soils; that the effect is even more pronounced under alkaline conditions; that the lack of nitrification of ammonium sulfate may be due more to the acid formed and that this is corrected by the application of  $\text{CaCO}_3$ . It is interesting to note that Kelley obtained active nitrification of dried blood in manured plot, which would increase the buffer content of the soil.

The one general criticism that can be applied to the soil method of studying nitrification is that the differences in the rate of nitrification may depend rather on the physical and chemical properties of the soil, than upon the number and activities of the bacteria originally present in the soil, as shown by Gerretsen (16) and others. The study of nitrification should, therefore, be carried out both in solution and in soil to give us a true picture of the phenomenon.

Differences in nitrate formation are not due to size or shape of container, quantity of soil used or depth of column, as long as the soil is loose, as pointed out by Gainey and Metzler (15).

Before taking up the experimental part of the work, it is not out of place to summarize briefly our knowledge of the influence of reaction on the activities of nitrifying bacteria. Various investigators have recorded the fact that the addition of lime to acid soils results practically in all cases in increased nitrifying activities. This is made clear by the fact that the optimum reaction for the activities of the nitrifying bacteria both of the nitrite and nitrate groups lies on the alkaline side of neutrality.

Meyerhof (35), in an exhaustive study on the oxidation of nitrifying bacteria found that the nitrate formers act, in solution, at reactions ranging between pH 5.6 and 10.3 with an optimum at pH 8.3 to 9.3. The optimum for the nitrite bacteria lies at pH = 8.8. Gaarder and Hagem (13) found the optimum for the growth of the nitrate bacteria to be at pH 7.0–7.2, and for nitrite bacteria at pH 7.8, the kind of buffer being of importance. The difference between their results and those of Meyerhof is explained either by the difference in method used, by an actual difference in races of bacteria or by the fact that Meyerhof studied the respiration of the organisms during a short period of time, while Gaarder and Hagem studied the growth of the organisms.

Gerretsen (16) found the acid limit for nitrification (using the solution method) to be at pH 3.9–4.5, depending on the origin of the nitrifying bacteria, those of acid soils being more adapted to acid conditions. The limiting alkaline reaction was found to be at pH 8.9–9.0, the buffer effect of  $\text{CaCO}_3$  and  $\text{Fe}(\text{OH})_3$  being important. The microorganisms resist also a greater degree of acidity in the soil than in solution.

When we add an ammonium salt to the soil for testing the nitrifying capacity of that soil, a further increase in acidity results, so that in the case of acid soils, particularly those with a low buffer content, the limiting reaction is soon reached. The amount of nitrate formed may then be limited by this final acidity rather than by the nitrifying activities of the soil as such, as pointed out above. It is interesting to record that soils containing scarcely any nitrate and capable of nitrifying ammonium salts only to a very small extent, usually nitrify

in solution, indicating the presence of nitrifying organisms. It is, therefore, reasonable to expect that the addition of lime to acid soil will result in an increased activity of the soil nitrifying flora, when ammonium salts are used in the test. Unfortunately, in the majority of studies on nitrification in acid soils, merely the lime requirement of the soil is recorded rather than the reaction expressed in terms of hydrogen-ion concentration. The former expressed not so much the actual acidity of the soil as its combining power with lime or buffer content. The greater lime requirement may indicate both a higher acidity, which is injurious to nitrification, or a higher buffer content of the soil, which may allow a greater accumulation of nitrates, or prove beneficial to nitrification as the test is commonly carried out. This method of expressing acidity is, therefore, valueless as far as the test for the nitrifying activities of the soil is concerned. There is no discrepancy between the statements of some of the earlier workers, Warrington (48, p. 42-76, 77-94) and Deherain (9) who thought that acid soils inhibited nitrification which takes place only in feebly alkaline media and may go on in soils deficient in lime, and some of the later workers, as White (49), Stephenson (40), who found that nitrification takes place in acid soils. It depends on the degree of acidity and method of measurement.

Hall, Miller and Gimingham (18) found that continuous treatment of a soil with ammonium sulfate will make the soil so acid as to greatly reduce nitrification. The presence of nitrates in the soil indicates that nitrification probably takes place in the presence of small isolated particles of calcium carbonate. Petit (37), Abbot and associates (1), Temple (44) and others soon reported active nitrification in acid or non-basic soils. Noyes and Conner (36) demonstrated that the amounts of nitrate present and the nitrifying power of untreated acid soils varied with the organic matter and total nitrogen rather than with the soil acidity.

According to the results presented by Stephenson (40) lime as such (in the form of carbonate) does not stimulate the activities of the nitrifying bacteria in the soil, as seen from the fact that no increase in nitrification of the soil's own nitrogen took place as a result of the addition of lime. Lime serves merely as a base for neutralizing the acid formed from the oxidation of ammonium sulfate used in carrying out the nitrification test. The nitrifying capacity of the same soil placed in a series of pots and treated with various amounts of  $\text{CaCO}_3$  was tested by adding 100 mgm. of ammonium sulfate to 100-gm. soil portions in tumblers. There was a progressive increase in the capacity of the soil to accumulate nitrates by increasing the lime treatment to 7 tons, further additions did not result in any appreciable increase. By adding acid to soil, a much greater repression of nitrification was obtained in a sandy than in a loam soil, due to the lower buffer content of the former, which will result in a greater hydrogen-ion concentration, with the same amount of acid than the loam soil. This again points to the absolute futility of testing the soil by the lime requirement methods when studying nitrification.

To point out the importance of the presence of basic materials, like calcium carbonate, for the neutralization of the acids formed in the process of nitrification, the work of Ames (3) may be cited. Ames found that in an acid silt loam, in the absence of  $\text{CaCO}_3$ , dried blood was nitrified to a greater extent than ammonium sulfate, the nitrates formed from the latter being less than in the untreated soil. When  $\text{CaCO}_3$  was added at the rate of 4000 parts per million, the nitrification of both forms of nitrogen was increased. With 8000 parts of  $\text{CaCO}_3$ , the formation of nitrate nitrogen from ammonium sulfate exceeded the amount produced from dried blood. Calcium phosphate can take the place of the carbonate in supplying a base for nitrification only to a limited extent for the very obvious reason that while the carbonate is brought into solution by the action of the acid at pH 6.0-7.0, which is optimum for the activities of the nitrifying bacteria, the phosphate goes mostly into solution at pH 3.0, much below the acid limit of their activities.

Fischer (10) found that the theoretical amount of lime (200 mgm. of  $\text{CaCO}_3$ ) required for the nitrification of ammonium sulfate (132.7 mgm.) was not sufficient to complete nitrification, but about 3.5 times the theoretical amount is required.

## EXPERIMENTAL

The same plots that were used for the study of bacterial numbers (46) and ammonification (47) were employed in this study. It is important to remember that 5A and 5B are the plots receiving 16 tons of stable manure per acre and minerals every year; 7A and 7B, no fertilizer at all; 9A, 320 lbs. of  $\text{NaNO}_3$  per acre and minerals; 11A and 11B,  $(\text{NH}_4)_2\text{SO}_4$  equivalent in nitrogen content to 320 lbs. of  $\text{NaNO}_3$  and minerals; 4A, 19A and 19B minerals only; and 18A, manure, nitrate and minerals. All the B plots were limed every 5 years at the rate of 2 tons  $\text{CaCO}_3$  per acre.

TABLE 1  
*Nitrification of ammonium sulfate in 100 gm. of soil*

PLOT NUMBER	TREATMENT*	NITRATE NITROGEN	INCREASE OVER SOIL	N (IN AMMONIUM SULFATE) NITRIFIED
		mgm.	mgm.	per cent
4A	Soil itself	0.90		
4A	$(\text{NH}_4)_2\text{SO}_4^*$	1.46	0.56	1.9
5A	Soil itself	1.4		
5A	$(\text{NH}_4)_2\text{SO}_4$	2.67	1.27	4.2
7A	Soil itself	0.7		
7A	$(\text{NH}_4)_2\text{SO}_4$	0.48	-0.22	0.0
9A	Soil itself	0.88		
9A	$(\text{NH}_4)_2\text{SO}_4$	3.03	2.15	7.2
11A	Soil itself	1.6		
11A	$(\text{NH}_4)_2\text{SO}_4$	0.95	-0.55	0.0
18A	Soil itself	0.81		
18A	$(\text{NH}_4)_2\text{SO}_4$	6.11	5.30	17.7
19A	Soil itself	0.9		
19A	$(\text{NH}_4)_2\text{SO}_4$	0.81	-0.09	0.0
7B	Soil itself	1.08		
7B	$(\text{NH}_4)_2\text{SO}_4$	7.54	6.46	21.5
11B	Soil itself	0.45		
11B	$(\text{NH}_4)_2\text{SO}_4$	4.80	4.35	14.5
19B	Soil itself	0.85		
19B	$(\text{NH}_4)_2\text{SO}_4$	9.39	8.54	28.47

\*  $(\text{NH}_4)_2\text{SO}_4$  treatment consisted of 30 mgm. of nitrogen as ammonium sulfate added to 100 gm. of soil and incubated for 30 days.

Samples were taken to a depth of 6 and  $\frac{3}{4}$  inches by means of a borer from 5 to 6 different parts of each plot, brought to the laboratory, well mixed and sieved through a sieve to remove the stones, coarse pebbles and organic matter. The soil was then placed, in 100-gm. portions, in tumblers, to which the proper amount of ammonium sulfate (in solution) or dried blood were added, well mixed and enough water to bring to two-thirds saturation. The tumblers were covered with glass plates and incubated at  $27^\circ\text{C}$ . for 30 days, unless otherwise stated. Moisture was added at weekly intervals to keep at optimum. At the end of the incubation period the soil was well mixed,

5 gm. were usually used for determining the hydrogen-ion concentration, by the colorimetric method, 50 gm. were placed in 500 cc. flasks, to which some CaO and distilled water was added and shaken for 10-15 minutes. The extract was then filtered and an aliquot portion evaporated to dryness and nitrates determined by the phenol-di-sulfonic acid method (Davis (8)).

The nitrates were always calculated back to the original 100 gm. of soil used and recorded as milligrams of nitrate nitrogen. In some cases 200 gm. of soil were used. The ammonia was then determined in one-half of the mixed soil by distilling with MgO. Two or three tumblers were usually used for each determination, but only the averages are recorded, due to the fact that the duplicates checked up fairly well.

The two unproductive plots 7A and 11A did not nitrify the ammonium sulfate at all; this is probably due to the high acidity of these plots; the limed plots 7B, 11B and 19B allowed a much greater accumulation of nitrates from

TABLE 2  
*Nitrification of soil, ammonium sulfate and dried blood*

PLOT NUMBER	SOIL ITSELF (100 GM.)		30 MGM. N AS $(\text{NH}_4)_2\text{SO}_4$ PER 100 GM. SOIL		1 GM. DRIED BLOOD (11.5% N) PER 100 GM. SOIL	
	Final reaction	NO <sub>3</sub> -N	Final reaction	NO <sub>3</sub> -N	Final reaction	NO <sub>3</sub> -N
	pH	mgm.	pH	mgm.	pH	mgm.
4A	5.3	1.2	5.0	1.9	5.7	11.8
5A	5.3	1.7	4.6	5.5	5.2	24.5
7A	4.8	0.6	4.8	0.5	6.0	11.7
9A	5.4	1.0	5.0	2.5	5.3	15.5
11A	4.0	1.0	3.8	0.4	5.0	12.5
18A	5.4	2.0	4.6	6.4	4.7	28.8
19A	5.2	1.3	4.9	1.2	5.2	13.7
7B	6.0	1.1	4.4	11.3	5.3	13.8
11B	5.4	1.2	4.8	5.0	5.1	20.0
19B	6.3	1.0	5.0	12.5	6.0	12.6

the ammonium sulfate than the corresponding unlimed plots without any reference at all to productivity but depending entirely on the initial reaction and buffer content of the plots. Where soil containing different amounts of lime and of a different reaction and buffer content are compared in their capacity of nitrifying ammonium sulfate, the results are entirely misleading. The accumulation of nitrates from ammonium sulfate depends upon the final reaction of the medium; the less acid the initial reaction and the greater the buffer content of a soil, the larger will be the amount of nitrate accumulated from ammonium sulfate before the final acid reaction is attained. These two factors prevent us from interpreting properly the results obtained from nitrification studies under those conditions. This is made even clearer in the following experiment, where the final reaction is also recorded.

The ammonium sulfate column in table 2 brings out the same differences as those found in table 1. The nitrate is formed from ammonium sulfate

until the reaction reaches a minimum somewhere between pH 4.4 and pH 5.0 and the total amount of nitrate is determined merely by the initial reaction and buffer content of the soil. The largest amounts of nitrate were formed from 7B and 19B, because of their relatively low initial acidity. Both of these plots are relatively unfertile since they received no nitrogen application for the last 15 years. The next greatest nitrification occurred in 5A and 18A, which are very productive plots and have high buffer contents due to the yearly applications of manure. Plots 7A and 11A, the most acid plots, produced even less nitrates than were formed in the test where nitrification of soil's own nitrogen took place.

The results with dried blood are distinctly different. The largest amounts were formed by 5A and 18A, the two manured plots; then come 9A and 11B, the plots receiving sodium nitrate and ammonium sulfate with lime, next in productivity to the manured plots. However, no such sharp differences are obtained with the dried blood, due to the fact that the ammonia formed from the decomposition of the dried blood neutralizes the acidity of the soil (7A and 11A) allowing nitrification to take place. No such lack of nitrification of 1 per cent of dried blood as observed by Kelley is found here, due to the fact that all these plots are sufficiently acid to combine with the ammonia formed so that it cannot injure the action of the nitrifying bacteria, while Kelley worked with less acid soils and perhaps less buffered, where the ammonia from the dried blood would readily become injurious.

One can readily recognize that ammonium sulfate would bring out much greater and more accurate differences between the different soils, if the rapid formation of a maximum acidity could be prevented. Various amounts of CaO were then added to several lots of the different soils, and after the soil was air dry, the nitrogen substances were added, as well as the proper amount of moisture.

Several important observations can be made from the data in table 3. Nitrification of ammonium sulfate is considerably increased by the application of lime, particularly in soil having a reaction of a pH less than 6.0; if an excess of lime in the form of CaO is added so that the reaction of the soil becomes equivalent to pH 8.0-8.2, nitrification of ammonium sulfate is injured, probably due to the toxic effect of free ammonia formed from the interaction of the oxide and sulfate. In the case of dried blood (using 1-per cent concentration), the injurious influence of the original reaction may set in even at pH 7.4, due to the fact that the reaction becomes quickly alkaline as a result of the ammonia formed from the decomposition of the dried blood; when the soil is richly buffered, the injurious influence does not set in as quickly. This was the reason why Lipman, Kelley and others found that dried blood (1 per cent concentration) does not nitrify in arid soils, poor in humus, which are apt to be of an alkaline reaction to start with.

The following experiment (table 4) deals with the same phenomenon, in a more extensive way.



Nitrification of ammonium sulfate in a well buffered soil (5A) of an acid reaction is greatly increased by the addition of CaO. However, in a poorly buffered acid soil (7A), the stimulating effect of CaO on nitrification is not so marked, and it may rapidly depress it altogether when used in excess. The CaO liberates the ammonia from the ammonium sulfate thus exerting a decided toxic effect upon the activities of the nitrifying bacteria. This toxic effect is marked also in well buffered soils (5B), when the reaction is made too alkaline. A similar phenomenon is observed in the nitrification of dried blood. Even as large a concentration as 1 per cent of dried blood is readily nitrified in well buffered soils (5A, 5B) and may even be stimulated by the addition of small amounts of CaO; in poorly buffered soils (7A, 7B), nitrifica-

TABLE 3  
*Nitrification of ammonium sulfate and dried blood in the soil, with and without CaO*

PLOT NUMBER	SOIL ITSELF (100 GM.)			SOIL + CaO*			30 MCM. N AS $(\text{NH}_4)_2\text{SO}_4$ IN 100 GM. SOIL			$(\text{NH}_4)_2\text{SO}_4 + \text{CaO}^\dagger$		
	Initial reaction	$\text{NO}_3\text{-N}$	Final reaction	Initial reaction	$\text{NO}_3\text{-N}$	Final reaction	Initial reaction	$\text{NO}_3\text{-N}$	Final reaction	Initial re- action	$\text{NO}_3\text{-N}$	Final re- action
	pH	mgm.	pH	pH	mgm.	pH	pH	mgm.	pH	pH	mgm.	pH
5A	5.4	2.0	5.4	7.7	3.0	7.4	5.4	3.2	4.6	7.2	16.0	4.4
5B	6.4	2.8	6.2	7.8	1.5	7.6	6.4	12.5	4.6	7.1	9.5	5.4
7A	4.8	0.5	4.8	7.9	0.9	7.9	4.7	0.21	4.2	7.6	1.8	7.2
7B	5.8	0.75	5.8	7.5	1.2	7.4	5.8	5.6	5.0	7.2	10.0	5.6

PLOT NUMBER	$(\text{NH}_4)_2\text{SO}_4 + \text{CaO}^*$			1 PER CENT OF DRIED BLOOD			DRIED BLOOD + CaO*					
	Initial reaction	$\text{NO}_3\text{-N}$	Final reaction	Initial reaction	$\text{NO}_3\text{-N}$	Final reaction	Initial reaction	$\text{NO}_3\text{-N}$	Final reaction			
	pH	mgm.	pH	pH	mgm.	pH	pH	mgm.	pH			
5A	7.7	34.0	4.5	5.4	29.8	5.2	7.4	40.6	4.8			
5B	7.8	14.0	5.6	6.4	20.6	5.8	7.6	25.6	6.6			
7A	8.2	Tr.	8.0	4.8	11.2	7.4	7.4	0.8	8.2			
7B	7.4	8.8	5.8	5.8	20.6	5.9	7.4	0.5	8.6			

\* 200 mgm. of CaO were used for 5A and 5B; 300 mgm. for 7A, and 100 mgm. for 7B.

† 100 mgm. of CaO were used for 5A and 5B; 200 mgm. for 7A and 50 mgm. for 7B.

tion of 1 per cent dried blood may or may not proceed rapidly, depending on the rapidity of ammonia formation. If the ammonia formation is at first slow, nitrification of dried blood may set in rapidly even in poorly buffered soil; if ammonia formation is rapid from the first and insufficient buffering agents are present, nitrification will be repressed. The addition of an excess of CaO even to well buffered soil will have the same effect.

The following experiment was carried out in a similar way by the use of a larger number of soils and results are given in table 5.

The previous observations hold true also for all the 11 soils obtained from the variously treated plots. The amount of nitrate formed from ammonium sulfate depends on the initial and final reaction of the soil and buffer content

of the particular soil. Plot 5B gave about 7 times as much nitrate as 5A. This was due entirely to the fact that the initial reaction of the former was pH 6.4 and of the latter only pH 5.4, while the final reaction was about pH 4.6-4.8. Plot 7B produced 12 times as much nitrate as 7A due to the fact that the initial reaction of 7B was pH 6.0 and of 7A only pH 4.8 which is near the maximum acidity. Plot 19B allowed only a little more than a half the ac-

TABLE 4  
*Influence of reaction upon nitrification of ammonium sulfate and dried blood\**

SOIL TYPE	SOURCE OF NITROGEN	CaO ADDED	INITIAL REACTION	FINAL REACTION	NH <sub>4</sub> -N	NO <sub>3</sub> -N
		mgm.	pH	pH		
5A	50 mgm. N as (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0	5.4	4.7		5.8*
		100*	7.0	4.6		16.2
		250	7.4	4.6		31.8
7A	50 mgm. N as (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0	4.8	4.7		0.8
		200	7.4	7.2		1.8
		300	7.8	7.8		Tr.
		500	8.6	8.6		Tr.
5B	50 mgm. N as (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0	6.4	4.6		37.2
		100	7.1	4.6		43.8
		250	8.0	7.4		5.8
5A	1 per cent dried blood	0	5.4	5.2	29.52*	45.4
		100	7.0	5.8	10.08	56.8
		250	7.6	7.8	48.56	2.6
7A	1 per cent dried blood	0	4.8	6.8	67.6	1.2
		200	7.4	8.2	62.4	0.8
		300	7.8	8.6	61.8	0.0
5B	1 per cent dried blood	0	6.4	5.5	39.4	16.2
		50	7.0	6.2	6.62	31.6
		250	7.8	6.4	9.82	27.0
7B	1 per cent dried blood	0	6.0	5.8	29.2	20.2
		50	7.2	7.8	49.4	0.5
		100	7.8	8.8	43.12	0.0

\* Quantities are given per 100 gm. of soil.

cumulation of nitrate in 5B, with approximately the same initial acidity, due to the higher buffer content of the second soil. When enough CaO is added to make the initial acidity approximately pH 7.2-7.4, nitrification of all soils is increased. Under these conditions the influence of the initial reaction upon the nitrifying capacity is eliminated. Plots 5A, 18A and 5B are the most fertile and give the highest amounts of nitrates. Plots 7A, 19A and 11A are the least fertile and give the lowest amounts of nitrates.

However, the use of CaO with ammonium sulfate brings about a rapid increase in the alkalinity of the soil which may often prove injurious to the action of the nitrifying bacteria. Table 6 is representative of the comparative influence of  $\text{CaCO}_3$  and CaO upon nitrification in the soil.

To be able to interpret nitrification results, it is important to know the course of nitrate formation in the soil as shown in table 7.

In view of the fact that the activities of the nitrifying bacteria are so much affected by the initial and final reaction of the soil used as a medium, all the soils should be brought first, by means of CaO or acid, to a certain reaction optimum for the activities of the organisms, like pH 7.0 to 7.2 and then tested for the nitrifying capacity; or they should receive a definite amount of  $\text{CaCO}_3$

TABLE 5  
*Influence of reaction upon the nitrification of ammonium sulfate and dried blood by 100 gm. of various soils*

PLOT NUMBER	SOIL ITSELF			50 MGM. N AS ( $\text{NH}_4$ ) $_2\text{SO}_4$		50 MGM. N AS ( $\text{NH}_4$ ) $_2\text{SO}_4$ + CaO*		1 PER CENT OF DRIED BLOOD			1 PER CENT OF DRIED BLOOD + CaO*		
	Final reaction		NO $_3$ -N	Final reaction		NO $_3$ -N	Final reaction	Final reaction		NO $_3$ -N	Final reaction		NO $_3$ -N
	pH	mgm.		pH	mgm.			pH	mgm.		pH	mgm.	
4A	5.6	1.4	2.5	4.8	2.5	4.9	34.2	5.4	38.1	21.6	7.9	56.7	0.8
5A	5.4	0.8	5.5	4.7	5.2	5.1	56.8	5.0	29.7	39.5	6.4	3.2	54.2
7A	4.8	0.3	1.3	4.8	0.8	7.4	0.6	7.0	47.7	0.5	8.2	42.2	0.0
9A	5.8	0.8	2.2	5.0	8.5	6.1	27.2	6.0	32.9	26.8	8.5	44.6	0.0
11A	4.6	0.8	0.8	4.6	0.2	6.2	19.2	6.9	64.1	0.8	7.8	48.3	0.0
18A	5.5	0.8	2.4	4.9	11.2	5.0	52.6	5.0	28.3	45.5	6.2	4.6	40.4
19A	5.4	0.8	1.1	4.9	2.2	6.1	12.4	6.6	47.8	12.8	8.2	39.2	0.0
5B	6.5	1.2	2.8	4.8	37.3	5.0	41.8	5.7	13.4	16.6	6.2	2.7	30.8
7B	6.0	0.8	1.5	4.9	9.6	5.1	33.1	7.2	37.9	2.4	7.9	35.6	0.0
11B	5.6	0.6	3.7	4.8	7.3	4.9	37.7	5.3	10.2	21.2	6.2	10.9	29.4
19B	6.2	1.2	3.2	4.9	20.2	5.0	29.2	7.0	35.8	0.8	7.8	35.2	0.4

\* The amounts of CaO used were:

250 mgm. for 5A, 7A, 11A, 18A

200 mgm. for 4A, 9A, 19A

100 mgm. for 5B, 7B, 11B, 19B

along with the ammonium salt, which will be just sufficient for the neutralization of the nitric and sulfuric acid formed, so as not to change the final reaction of the medium. Under these conditions we can study the influence of the sum total of the soil, including the physical, chemical and bacteriological conditions upon its nitrifying capacity. For the nitrification of organic matter, a smaller concentration of dried blood should be used, as suggested by Kellev (25). These methods have their advantages and disadvantages.

The advantages of the method of adjusting the initial reaction consist in testing the optimum potential nitrifying capacity of the soil, by bringing all the soils to the same reaction basis; thus the original and limiting acidity factors

TABLE 6  
Influence of CaO and CaCO<sub>3</sub> on the nitrification of 50 mgm. of N as (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> in 100 gm. of soil after 28 days' incubation

PLOT NUMBER	SOIL ALONE		SOIL AND VARYING AMOUNTS OF CaCO <sub>3</sub>									
			80 mgm.		160 mgm.		400 mgm.		800 mgm.		2 gm.	
	Initial re-action	NO <sub>3</sub> -N	Initial re-action	NO <sub>3</sub> -N	Initial re-action	NO <sub>3</sub> -N	Initial re-action	NO <sub>3</sub> -N	Initial re-action	NO <sub>3</sub> -N	Initial re-action	NO <sub>3</sub> -N
	pH	mgm.	pH	mgm.	pH	mgm.	pH	mgm.	pH	mgm.	pH	mgm.
5A	5.4	5.2	6.3	15.2	6.8	24.8	7.2	36.4	7.5	46.4	7.6	54.2
7A	4.8	0.05	...	....	...	....	...	....	...	....	...	....
11A	4.4	0	...	....	6.0	7.6	6.9	5.6	7.6	2.4	...	....
19A	5.4	Tr.	6.4	1.2	7.0	5.9	7.5	1.8	7.7	0.5	7.8	0.6
5B	6.6	11.3	7.3	28.6	7.5	8.9	7.6	7.5	7.6	8.4	...	....
7B	6.4	4.4	...	....	...	....	...	....	...	....	...	....
11B	6.2	2.8	6.5	8.8	7.2	19.5	7.5	15.4	7.7	16.8	7.8	15.9

PLOT NUMBER	SOIL AND VARYING AMOUNTS OF CaO									
	40 mgm.		80 mgm.		200 mgm.		400 mgm.		800 mgm.	
	Initial re-action	NO <sub>3</sub> -N	Initial re-action	NO <sub>3</sub> -N	Initial re-action	NO <sub>3</sub> -N	Initial re-action	NO <sub>3</sub> -N	Initial re-action	NO <sub>3</sub> -N
	pH	mgm.	pH	mgm.	pH	mgm.	pH	mgm.	pH	mgm.
5A	6.2	14.2	6.4	19.6	6.8	36.8	7.1	48.6	7.9	16.8
7A	...	....	6.0	1.2	6.8	Tr.	...	....	...	....
11A	...	....	5.8	2.9	6.5	0.6	7.4	0	7.9	0
19A	6.5	2.6	6.6	8.5	6.8	1.4	7.6	0.42	8.2	0.12
5B	6.8	27.9	7.1	17.3	7.4	8.4	7.8	6.2	...	....
7B	6.8	6.4	7.3	4.2	7.7	3.9	8.0	0.3	...	....
11B	6.5	9.1	6.7	17.2	7.0	20.8	7.7	5.4	8.4	0.5

TABLE 7  
Course of nitrate formation from 30 mgm. N as (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and from 1 per cent of dried blood in 100 gm. of soil

PLOT NUMBER	NO <sub>3</sub> -N FROM AMMONIUM SULFATE			NO <sub>3</sub> -N AND NH <sub>3</sub> -N FROM DRIED BLOOD								
	After 7 days	After 20 days	After 42 days	After 7 days			After 20 days			After 42 days		
				Reaction	NH <sub>3</sub> -N	NO <sub>3</sub> -N	Reaction	NH <sub>3</sub> -N	NO <sub>3</sub> -N	Reaction	NH <sub>3</sub> -N	NO <sub>3</sub> -N
	mgm.	mgm.	mgm.	pH	mgm.	mgm.	pH	mgm.	mgm.	pH	mgm.	mgm.
5A	1.7	4.2	8.6	6.8	50.7	8.9	5.6	57.9	43.5	5.2	47.6	71.0
7A	0.2	0.4	0.7	7.4	38.1	1.6	8.1	64.8	3.8	7.6	57.5	6.2
11A	0.3	0.3	0.3	6.9	44.1	3.6	7.8	81.6	4.9	7.8	86.2	5.0
5B	5.8	12.0	25.3	6.2	41.9	21.0	5.9	33.5	57.0	5.0	34.3	75.5
7B	1.5	7.2	13.6	7.6	45.2	4.9	7.5	44.7	5.9	7.3	41.0	5.5
11B	1.8	5.2	10.8	7.6	56.7	10.7	6.9	55.9	16.0	6.6	54.9	29.9

are eliminated, and a basis for comparison with neutral or faintly alkaline soils is obtained. Under these conditions we could use a test of the nitrifying capacity of the soil with both dried blood (0.1 or 0.25 per cent) and ammonium salts. The chief disadvantage of this method is that by adding lime to acid soils, sufficient to bring it to a pH of 7.0, we would have to use CaO, thus actually changing the physical and chemical condition of the soil as pointed out by Hutchinson and MacLennan (21), who found CaO to cause a partial sterilization of the soil, while  $\text{CaCO}_3$  does not. It is also important to note that CaO causes a considerable increase in the hydroxyl-ion concentration in the soil, followed by a gradual decrease, as shown by Hoagland and Christie (20):  $\text{CaCO}_3$  does not cause an appreciable change in reaction of neutral soils.

The chief advantage of the method of using a theoretical amount of  $\text{CaCO}_3$  sufficient to neutralize all the acid formed from the nitrification of the  $(\text{NH}_4)_2\text{SO}_4$  consists in the fact that, by this method, we can study the nitrifying capacity of the soil as such. At the same time there is enough basic material to prevent abundant acid formation from the oxidation of the ammonium salt so as not to produce in the more acid and poorly buffered (humus poor) soils a limiting acid reaction which will stop the activities of the nitrifying bacteria. However, as pointed out by Fischer (10), a theoretical amount of  $\text{CaCO}_3$  (200 mgm. for 132.2 mgm. ammonium sulfate) is not sufficient to neutralize completely all the acid formed, and we have to use 3.5 times the theoretical amount of  $\text{CaCO}_3$ . The disadvantage of this method consists in the fact that the oxidation of the ammonium salt and nitrate formation is slow and accumulative, while the  $\text{CaCO}_3$  or CaO added in the beginning of the test, will produce an initial change in the reaction of medium, the carbonate less than the oxide, which will make conditions not strictly alike for the different soils. The use of a small concentration of dried blood (0.1 to 0.25 per cent) recommends itself also as of practical value, in view of the fact that the small amounts of ammonia formed will not be sufficient to change the reaction even of neutral or slightly alkaline soils to a point where free ammonia would be given off, unless in very alkaline soils. Both of these methods recommend themselves as more scientific than those which have been commonly used in the past.

Table 8 gives a summary of a typical experiment on nitrification in solution [medium no. 29 given by Fred (11)], in pure sand, moistened with the same solution, nitrification of the soil's own nitrogen, of small amounts of dried blood in the soil and of ammonium sulfate without and with CaO and  $\text{CaCO}_3$ .

The solution method shows definite differences, and it may, therefore, be used to great advantage. The use of pure sand, however, presents certain advantages over the solution method, particularly since more pronounced differences are obtained. On further study, this method has been modified as follows:

One hundred grams of sand, 210 mgm.  $\text{CaCO}_3$  and 15 cc. of a medium containing 2.0 gm.  $\text{K}_2\text{HPO}_4$ , 1 gm.  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.4 gm.  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  in 1000 cc. of water, are placed in 250-cc.

Erlenmeyer flasks, well mixed, plugged with cotton and sterilized, at 15 lbs. pressure for 1 hour. The ammonium sulfate is then added in solution, usually 3 or 5 cc. of water containing 30 mgm. of nitrogen. For inoculation 10-gm. portions of soil are used. The flasks are well shaken to obtain proper uniformity and incubated at 27-28°C. for 30 days. Either the solution or the sand method is used. The sand method has been found to give more uniform results.

TABLE 8  
*A summary of results from methods of studying nitrification\**

PLOT NUMBER	NITRATE NITROGEN DETERMINED																			
	In solution with 10 gm. soil inoculum		In sand with 10 gm. soil inoculum†		In soil + 0.1 per cent of dried blood			In soil + 0.25 per cent of dried blood			30 mgm. N as (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>		30 mgm. N as (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> + 200 mgm. CaCO <sub>3</sub>		30 mgm. N as (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> + 700 mgm. CaCO <sub>3</sub>		30 mgm. N as (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> + CaO with initial reaction adjusted to pH 7.0			
	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	pH	mgm.	pH	mgm.	pH	mgm.	pH	mgm.	pH	mgm.	pH	mgm.	pH	mgm.
5A	0.76	7.80	10.70	2.50	1.64	10.40	5.1	24.40	4.8	5.20	4.6	24.40	6.9	34.80	6.9	28.20				
6A	.....	.....	.....	1.98	1.60	9.60	4.9	25.20	4.8	4.30	4.5	31.20	7.0	33.60	6.7	28.00				
6B	.....	.....	.....	1.14	3.00	9.40	5.3	25.30	4.9	11.60	5.9	30.90	6.8	32.60	6.0	23.30				
7A	0.15	0.21	0.22	0.38	0.72	3.60	5.3	7.20	4.6	0.33	6.2	7.60	7.8	2.80	7.5	4.40				
7B	0.38	1.44	1.04	0.60	1.20	10.80	5.0	18.30	4.8	6.20	6.2	16.95	7.3	20.60	5.1	17.70				
11A	0.46	0.44	0.42	0.84	0.96	4.20	4.6	5.00	4.2	0.76	5.6	3.10	7.8	5.20	7.2	6.80				
11B	0.84	5.70	13.50	0.94	1.52	10.60	4.8	21.20	4.8	3.80	5.2	26.10	7.3	27.50	6.05	22.00				

\* All cultures, except one-half of the group containing 0.1 per cent of dried blood, were incubated for 28-30 days at 28°C. Nitrate is given on the basis of milligrams of nitrogen as nitrate in 100 gm. of soil; pH is final.

† 100 gm. of pure washed quartz sand placed in 250-cc. Erlenmeyer flasks, moistened with 5 cc. of distilled water and sterilized for 2 hours at 15 lbs. pressure, with 200 or 700 mgm. CaCO<sub>3</sub>. 10 cc. of the medium used for the solution studies containing 30 mgm. of nitrogen as (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and sterilized separately, was added to each flask.

‡ The following amounts of CaO were used for 100 gm. of soil to bring the reaction of the soil to approximately pH 7.0-7.2:

Plot No.	Mgm. CaO	Plot No.	Mgm. CaO
5A	500	7B	50
6A	500	11A	375
6B	100	11B	250
7A	250		

Nitrification of the soil's own nitrogen gives important information. Nitrates are determined in the soil, soon after sampling. One hundred-gram portions of the soil are incubated in glass covered tumblers with optimum moisture (60 per cent of moisture-holding capacity) for 30 days. The nitrates are always determined in the moist soil, without previously drying it, due to the fact that the process of drying greatly increases the nitrate content of the soil, depending on the method of drying and type of soil.

Nitrification of ammonium sulfate in soil is best carried out in two ways: 1. The common method is to add 30 mgm. of nitrogen as ammonium sulfate to 100 gm. of soil and incubate with optimum moisture for 30 days. The nitrate formation under these conditions will give the maximum nitrate-holding capacity of the soil, or soil reaction and buffer content as influencing nitrate accumulation. 2. In addition to the ammonium sulfate 210 mgm. of calcium carbonate may be added to the soil. This allows us to study nitrification of ammonium salts under optimum conditions of reaction. In the case of alkali soils, this method may be superfluous.

It is also important to gain information on the nitrification of organic nitrogenous materials added to the soil. With an incubation period of 10 or 15 days at 27–28°C. 0.1 per cent of dried blood may be used. By using 0.25 per cent of dried blood, incubating 15 days, then determining the final reaction (pH value), ammonia content and nitrate, valuable information is gained.

By combining the information obtained from the study of nitrification by the five different methods, we can thus gain a proper picture of the process of nitrification in the soil and its bearing on soil fertility.

A detailed study of the correlation between results obtained from nitrification studies, numbers of microorganisms and crop productivity of the soil will be given in the following paper of this series.

#### SUMMARY

1. Nitrification of ammonium sulfate or other inorganic salts of ammonia in soils having different reactions cannot be used as a basis for comparison. This is due to the fact that the amount of nitrate accumulated in the soil, as commonly carried out under laboratory conditions, will depend on the initial reaction of the soil, buffer content and final reaction more so than on the bacteriological activities: less acid soils will allow a greater accumulation of nitrates than the more acid soils, under a given set of conditions.

2. In the oxidation of ammonium sulfate, nitric and sulfuric acid are formed. These acids increase the hydrogen-ion concentration of the soil, till a point is reached which becomes injurious to the activity of the nitrifying bacteria. In well buffered soils, such as those receiving large applications of organic matter, greater amounts of acid can be formed, before the injurious reaction is attained than in poorly buffered soils.

Nitrate accumulation from ammonium sulfate in the soil stops when the reaction has reached pH 4.4–4.8. The greater the buffer content of the soil the greater will be the amount of nitrate accumulated, even if the initial reaction is the same.

3. Nitrification of ammonium sulfate in the soil should be carried out, in laboratory studies, in the presence of sufficient basic material to neutralize the acids formed from the oxidation of the ammonium salt.  $\text{CaCO}_3$  is to be

preferred to CaO, since the latter tends to change rapidly the reaction of the soil and, therefore, bring about various uncontrolled chemical, physical and biological changes.

4. The nitrification of dried blood, in concentrations commonly employed, namely one per cent, is not a good test for comparing different soils. This is due to the different reactions involved in the transformation of dried blood, particularly in alkaline and poorly buffered soils. The rapid decomposition of the dried blood brings about an abundant formation of ammonia, which is not sufficiently neutralized by acids or buffering agents in alkaline and poorly buffered soils. The free ammonia has an injurious action upon the activities of the nitrifying bacteria.

5. Either low grade nitrogenous materials should be used or a low concentration of high grade materials, for the study of nitrification of organic materials in the soil.

6. To get a thorough idea of the nitrifying capacity of the soil and have a basis for comparing the nitrification of different soils, no one single method is sufficient but a combination of the following methods is recommended, all of which should be used for each soil and each of which gives some information necessary to obtain a complete picture of nitrification in the soil.

*a.* Nitrification in solution, using 10 per cent of soil for inoculation, as recommended by Remy, Löhnis and others. The information obtained is valuable for a knowledge of the nitrifying flora of the soil, and the influence of the constituents of the particular soil upon the nitrifying bacteria, when studied under standard laboratory conditions. The use of pure sand is to be preferred to the solution method: 100 gm. of pure washed sand + 210 mgm.  $\text{CaCO}_3$  + 15 cc. of mineral solution placed in plugged flasks and sterilized in the autoclave. Three to five cubic centimeters of ammonium sulfate solution containing 30 mgm. of nitrogen and sterilized separately are added and each flask inoculated with 10 gm. of soil to be tested.

*b.* Nitrification of soil's own nitrogen. A definite amount of soil (100 gm.) kept in the laboratory for a definite length of time (30 days) at a definite temperature (25–28°C.), under optimum moisture conditions, will give us information on the forms of nitrogen present in the particular soil and the speed with which they are transformed into nitrates and thus made available for plant growth.

*c.* Nitrification of ammonium sulfate in the soil. By using a definite amount of nitrogen (30 mgm. in 100 gm. of soil), in the form of ammonium sulfate, and standard period of incubation, we get, from the amount of nitrate formed, an index on the buffering capacity of the soil in relation to nitrification. The final reaction should always be recorded.

*d.* Nitrification of ammonium sulfate in the presence of a theoretical amount of  $\text{CaCO}_3$  [210 mgm. for 30 mgm. N as  $(\text{NH}_4)_2\text{SO}_4$ ] necessary to neutralize all the acid formed from the complete oxidation of the ammonium sulfate into nitric and sulfuric acid. This gives an index of the nitrifying capacity of



the soil under optimum reaction conditions and forms an excellent basis for comparing nitrification with other soil bacteriological activities.

*e.* Nitrification of organic nitrogenous materials. If high grade materials are used (dried blood), 0.1-per-cent concentration should be used and a brief incubation period (10-15 days); 0.25-per-cent concentration and a period of incubation of 15 or 15 and 30 days gives important supplementary information.

By giving a definite weight to the information obtained by each of these five methods, a true picture of nitrification in soil may be obtained.

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## AERATION METHOD FOR DETERMINING AMMONIA IN ALKALI SOILS

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In the investigation of bacterial activities in alkali soils at this station, it was imperative that a rapid method be found for the determination of ammonia. A satisfactory method for the determination must meet three requirements: First, all the ammonia in the soil must be removed and any added portion of ammonia must be fully recovered; second, organic nitrogenous material in the soil must not be broken down into compounds which yield ammonia; and third, the method must permit one to make several determinations within a reasonable length of time. Some of the methods which have been described prove impracticable because of complicated apparatus or the excessive time required for a complete determination. The copper flask distillation over magnesium oxide is quite extensively used, but cannot be applied to soils containing alkali salts because of the fact that the organic matter of the soil is attacked by the alkali and ammonia is split off. Potter and Snyder (5) have described a method which overcomes this difficulty. Their method calls for the use of a 25-gm. sample of soil and requires from 15 to 19 hours aeration at room temperature to complete a set of determinations. Davisson (1) and his associates have described an aeration method which is applicable to a larger sample and which requires but 2.5 hours for a determination. The objection to their method, however, is that an extract of the soil is used for the determination and not the soil itself, and the apparatus is somewhat complicated.

Matthews (4) describes an aeration method for the determination of ammonia in soils in which a 25-gm. sample is used and the aeration continued 6 hours at the rate of 300 liters per hour. He states that the time may be reduced to 3 hours in many cases. The apparatus is expensive and difficult to manipulate. Russell (6) tested several methods and applied heat to the aeration process.

Of the several methods reviewed, it seemed that the aeration method as described by Potter and Snyder was the most promising for the determination of ammonia in ammonification experiments with alkali soils if modifications could be made which would allow the complete determination of ammonia to be made on 100 gm. of soil in much less time than 15 to 19 hours.

A number of experiments were undertaken in which 100 gm. of soil were

used and in which an effort was made to hasten the removal of ammonia from the soil by increasing the temperature of the soil mixture during the aeration. In addition, experiments were carried out to show the effect of the increased temperature on the organic matter in the soil in the presence of definite amounts of alkali. The results are of especial interest to the biochemist.

#### METHOD

The aeration method for the determination of ammonia in urine was originally outlined by Folin (2), and the method applied to other solutions by Kober (3). Modifications were made by Potter and Snyder in their adaptation of the method to the determination of ammonia in soils. The apparatus has been described by them but a further description is briefly given. Figure 1 shows a cut of a unit of the aeration apparatus together with the trough used in heating the flasks during aeration.

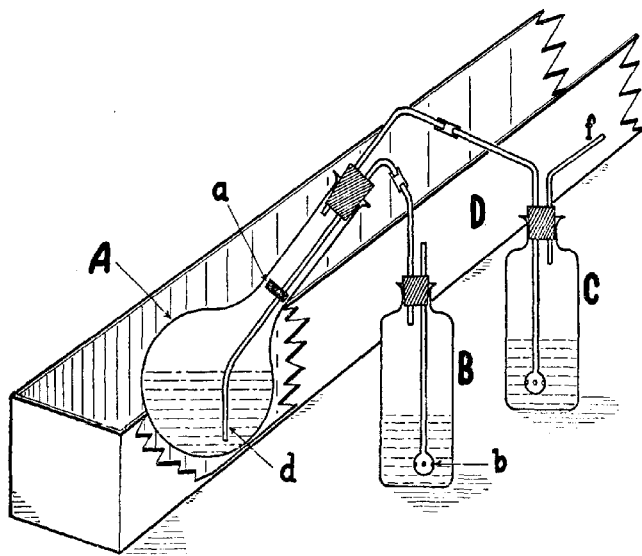


FIG. 1. AERATION APPARATUS

- |                                     |                                       |
|-------------------------------------|---------------------------------------|
| A. 800 cc. Kjeldahl flask           | a. Section of rubber stopper          |
| B. Dilute $\text{H}_2\text{SO}_4$   | b. Bulb for distributing air current  |
| C. Standard $\text{H}_2\text{SO}_4$ | d. Air inlet tube                     |
| D. Trough for hot water             | f. To another section or suction pump |

The Kjeldahl flask (fig. 1) contains 100 gm. of the soil to be analyzed, approximately  $\frac{1}{4}$  gm. of sodium carbonate, 0.5 cc. paraffin oil, and approximately 300 cc. of ammonia-free water. When suction is applied at *f* a stream of washed air goes through the flask of soil suspension, thence through the flask of standard acid where the ammonia is caught. It may then be led through additional flasks of soil and standard acid, and thence into the suction pump. The section of rubber stopper *a* over the tube *d* prevents splashing into the neck of

the Kjeldahl flask. The trough *D* is filled with water which is maintained at a temperature of 75°C. It will be found that if the trough is six to eight feet in length a stream of hot water near the boiling point can be passed in at one end and out at the other and in this way maintain a temperature of 75 to 80°C. Since the aeration process has a tendency to constantly lower the temperature it is noted that the temperature at one end of the trough is higher than at the other end. Determinations at either end of the trough in this case will check provided the temperature at the cooler end is not below 75°C. In this work no differences were obtained between temperatures of 75 and 80°C., but temperatures below 75°C. gave irregular results.

To overcome the difficulty of maintaining a uniform temperature throughout the trough, the apparatus shown in figure 2 was designed, in which steam is used for heating the water. The rush of steam through the pipe *c* creates a suction which draws the water in at the side neck *b*, forces it through *c*, and thus back to *b* again. The pipe is placed in the bottom of the trough to one side and is not in the way of the Kjeldahl flasks. By this means the water may be held at the temperature desired. A union joint is placed in front of the steam valve which provides an easy means of disconnecting the apparatus when not in use.

The temperature of the water in the trough and the rate of air flow are the most essential factors in the rapid and complete removal of the ammonia from soils by this method. If the temperature is below 75°C. a longer time is required to remove the ammonia. The air flow must be regulated at such a rate that the soil in the flask is constantly held in suspension.

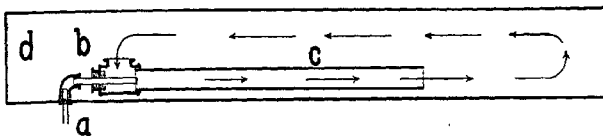


FIG. 2. HEATING ARRANGEMENT

- |               |                  |
|---------------|------------------|
| a. Steam pipe | c. One inch pipe |
| b. Side arm   | d. Trough        |

The end of the tube *d* (fig. 1) must be placed the proper distance from the bottom of the flask or the soil will settle regardless of the air flow. This distance was found to be about .25 inch. The section of rubber stopper assists in holding the tube in place and in addition prevents the contents of the flask from being mechanically carried into the neck. No estimate was made of the amount of air used in these determinations.

The suction was obtained by means of a Crowell vacuum pump driven by an electric motor. A condenser was placed between the last flask and the pump to remove the steam and thus prevent its condensing in the pump cylinder.

In setting up the apparatus it is well to arrange the Kjeldahl flasks in a slightly inclined position. The receiving bottles may then be arranged in a row by the side of the trough. The number of determinations that can be made at one time is limited only by the capacity of the vacuum pump. In this work six to ten determinations were made at one time, this being the maximum load the pump would pull and keep the soils well agitated.

The amount of acid chosen for the receiving flask must be in excess of the probable amount of ammonia present in the soil. Care must be exercised to have an excess in order to avoid complete neutralization, in which case ammonia will be carried into the next flask. In this work varying amounts of  $\frac{1}{4}$  N acid were used and the volume in the receiving flask brought to approximately 125 cc. with distilled water. At the end of the aeration process the volume will be found to have increased by 50-75 cc. due to condensation. The contents were then carefully transferred to 500-cc. Erlenmeyer flasks and boiled on a hot-plate until the volume

decreased by approximately one-third in order to remove volatile compounds liberated from the soil which interfere with a sharp end-point in titrating. When cool the excess acid was determined by titration using methyl red as indicator.

Davison and his associates state that the absorption of ammonia by this process is not complete. Our results, and likewise those of Potter and Snyder, indicate that all the ammonia is absorbed. Care should be taken in preparing the Folin tubes to make all the holes very small and of the same size. This gives small bubbles of air which are evenly distributed throughout the receiving flask. If the holes are not uniform some loss from splashing may result.

#### RESULTS

All experiments reported were made with a neutral silt loam soil of average fertility.

No attempt is made to present all the data obtained but only a few experiments to demonstrate the relative merits of the method. Many such experiments were made. The results obtained indicate clearly that the method may well be applied to soil biology. In the first series of experiments 5 gm. of magnesium oxide and 2 gm. of sodium carbonate were used to liberate the ammonia. The results obtained were in close accord with those obtained by the copper flask method and added quantities of ammonia were fully recovered. Later experiments demonstrated that 2 gm. of sodium carbonate was sufficient to liberate the ammonia. The magnesium oxide was omitted thereafter. Many experiments were made to establish the time necessary for complete removal of the ammonia. The results indicated clearly that 1 hour at 75°C. was sufficient. It is recommended, however, in routine analyses that the time be extended to 1.5 hours. It is quite probable that in the majority of determinations the ammonia was completely liberated within the first half hour. Amounts of sodium carbonate varying from 1 to 10 gm. were used, the results indicating that 2 gm. is sufficient to liberate the ammonia but that 10 gm. is not too great an excess.

#### *Experiment 1*

One-hundred-gram samples of soil received 43 mgm. nitrogen as ammonium sulfate and varying amounts of sodium carbonate. They were then aerated 1.5 hours. The results shown in table 1 indicate that 2 gm. of sodium carbonate liberated the ammonia from 100 gm. of soil when aerated 1.5 hours at 75°C. No difference is noted between 2 and 6 gm. of the carbonate, which indicates that no decomposition of the soil organic matter took place. This experiment was repeated aerating 1 hour. The results were in close accord with those shown in table 1 and indicate that an aeration of one hour is sufficient.

#### *Experiment 2*

Dried blood, glycine, alanine and asparagin were added to 100-gm. samples of the soil in definite amounts and the ammonia determined by aerating 1

hour. Dried blood, Witte's peptone, and asparagin were added to other samples along with 30 mgm. of nitrogen as ammonium sulfate. All samples received 10 gm. of sodium carbonate in the determination. Particular atten-

TABLE 1  
*Effect of sodium carbonate on the recovery of ammonia by aeration for 1.5 hours at 75°C.*

NUMBER	SODIUM CARBONATE	NITROGEN AS $\text{NH}_4^*$	AVERAGE
	gm.	mgm.	mgm.
1	1.0	38.8*	41.84
2	1.0	41.2	
3	1.0	40.5	
4	1.0	43.6	
5	1.0	45.1	
6	2.0	42.5	43.65
7	2.0	44.1	
8	2.0	43.8	
9	2.0	43.7	
10	2.0	44.2	
11	2.0	43.6	
12	3.0	44.4	43.88
13	3.0	43.7	
14	3.0	44.1	
15	3.0	42.8	
16	3.0	44.4	
17	3.0	43.9	
18	4.0	44.3	43.48
19	4.0	43.8	
20	4.0	43.4	
21	4.0	43.7	
22	4.0	42.2	
23	5.0	43.8	43.92
24	5.0	44.6	
25	5.0	44.8	
26	5.0	43.0	
27	5.0	43.4	
28	6.0	43.7	43.85
29	6.0	44.4	
30	6.0	44.0	
31	6.0	43.3	

\* Each sample received 43 mgm. of nitrogen as ammonium sulfate.

tion was paid to the temperature of the water, keeping it around 85°C. The results shown in table 2 indicate that none of the compounds under test were decomposed to yield ammonia by the aeration process. The sample of soil containing the peptone yielded 3.7 mgm. more nitrogen than was added as



ammonium sulfate. The peptone was found to contain 2.5 mgm. nitrogen as ammonia when determined by distillation over magnesium oxide. These results indicate that soil organic matter is not decomposed by this method to yield ammonia in ammonification experiments.

TABLE 2  
*Effect of aeration for 1 hour at 35°C. on organic compounds*

NUMBER	TREATMENT OF SOIL	NITROGEN AS $\text{NH}_3$	AVERAGE
		mgm.	mgm.
1	10 gm. $\text{Na}_2\text{CO}_3$ 1 gm. dried blood	0.6	0.7
2		0.7	
3		0.5	
4		0.9	
5	10 gm. $\text{Na}_2\text{CO}_3$ 1 gm. dried blood and 30 mgm. N. as $(\text{NH}_4)_2\text{SO}_4$	30.7	31.1
6		30.5	
7		31.5	
8		31.0	
9		31.3	
10		31.6	
11	10 gm. $\text{Na}_2\text{CO}_3$ 2.5 gm. Wittes peptone and 30 mgm. N. as $(\text{NH}_4)_2\text{SO}_4$	34.4	33.7
12		32.9	
13		33.8	
14	10 gm. $\text{Na}_2\text{CO}_3$ 14 mgm. N. as Asparagin and 30 mgm. N. as $(\text{NH}_4)_2\text{SO}_4$	30.8	31.3
15		31.7	
16		31.3	
17	10 gm. $\text{Na}_2\text{CO}_3$ 0.1 gm. glycine	1.4	1.45
18		1.5	
19	10 gm. $\text{Na}_2\text{CO}_3$ 0.12 gm. alanine	1.1	1.0
20		0.9	
21	10 gm. $\text{Na}_2\text{CO}_3$ 0.188 gm. asparagin	1.3	1.4
22		1.5	

### Experiment 3

One-hundred-gram samples of soil received 2 gm. of finely ground dried blood and 26 gm. of water and were then incubated 1 week at 28°C. At the end of this period ammonia was determined by the method above described, aeration being continued 1 hour. The results shown in table 3 indicate that the yields of ammonia from the samples agree very closely. The average yield of ammonia when 2 gm. of sodium carbonate are used in the determinations is greater than the average where 4 or 6 gm. are used. No significance can be attached to their slight variation since the ammonia content of the

samples varies slightly. A variation of 1-2 mgm. of nitrogen as ammonia is negligible in ammonification experiments where an amount as high as 120 mgm. is involved. The results clearly indicate that only the ammonia in the soil is liberated by the aeration process and that no decomposition of the organic matter to yield ammonia is taking place. These data also indicate that 2-6 gm. of sodium carbonate may be used in the determination. This is a matter of considerable importance since it eliminates weighing the carbonate in routine determinations.

TABLE 3

*Effect of varying amounts of sodium carbonate on ammonia content with aeration for 1 hour at 75°C.*

NUMBER	SODIUM CARBONATE	NITROGEN AS $\text{NH}_3$	AVERAGE
	gram	mgm.	mgm.
1	2.0	121.9	121.5
2	2.0	117.9	
3	2.0	120.7	
4	2.0	124.7	
5	2.0	123.6	
6	2.0	120.4	
7	4.0	119.0	121.0
8	4.0	117.0	
9	4.0	122.5	
10	4.0	124.2	
11	4.0	121.6	
12	4.0	122.0	
13	6.0	121.4	120.4
14	6.0	119.8	
15	6.0	123.0	
16	6.0	119.4	
17	6.0	119.9	
18	6.0	119.2	

## SUMMARY

1. Complete aeration of the ammonia from 100 gm. of soil was obtained in 1-1.5 hours at a temperature of 75 to 85°C.
2. No ammonia is formed from the organic matter in soil in the presence of alkali concentrations and temperatures used in these experiments.
3. The aeration method can be used for the determination of ammonia in ammonification experiments with alkali soils.

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## METHOD FOR ESTIMATING ADSORBED BASES IN SOILS AND THE IMPORTANCE OF THESE BASES IN SOIL ECONOMY<sup>1</sup>

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A part of the bases (calcium, magnesium, potassium, sodium, and ammonium) which may occur in the clay-humus complex of the soil is present in a replaceable or exchangeable form. It is possible to demonstrate this fact by treatment of the soil with a solution of a salt of one of these bases (for example with ammonium chloride). We then find a replacement of calcium, magnesium, potassium, or sodium of the soil by equivalent proportions of the ammonium from the solution. The replacement process is reversible; ammonium ions from the solution are capable of replacing Ca, Mg, K, and Na ions from the soil; but these in turn can also replace the soil ammonium to a certain extent. As soon as equilibrium is established we find in both the soil and the solution  $\text{NH}_4$ , Ca, Mg, K, and Na ions. Solutions of  $\text{NaNO}_3$ ,  $\text{CaSO}_4$ , etc, behave in a similar manner.

Experiments here reported show that equilibrium is very quickly attained in this "replacing" process. This fact indicates that here we are dealing with a reaction between the solution and the easily accessible or surface particles of the clay-humus complex. If the bases on the interior of the adsorbing particles were concerned either partly or wholly in this reaction equilibrium would only be reached slowly, because diffusion occurs very slowly in solid bodies. Other phenomena would also make it appear that the particles which take part in this process are present in the ionic form. We may, therefore, consider that at the boundary surface between the soil as the solid phase and of the soil solution as the liquid phase, replaceable cations occur,—that is, replaceable cations are adsorptively bound to the adsorbing soil particles. The idea then occurs that the exchangeable cations and the adsorptively-bound cations may be identical.

Explanations of this base-adsorption are to be sought in chemical relations: chemical compounds are formed between the adsorbed bases (Ca, Mg, K, Na, and  $\text{NH}_4$ ) and the clay- and humic-acids of the soil. The fact that only the molecules in the surface layer react to form these chemical combinations characterizes the reaction as an adsorption process.

<sup>1</sup> For original paper, tables and references, see *Verslagen van Landbouwkundige Onderzoekingen der Rijkslandbouwproefstations*, 1920, no. 24, p. 144-250.

Since the chemical combination only occurs at the surface of the adsorbing clay-humus particles we can well understand that, from calculations based on the mass of the whole particle, no combination occurs in simple stoichiometric proportions as is the case in ordinary chemical reactions of this kind. This difference in extent of reaction between adsorption-combinations and others of a purely chemical nature gradually becomes less as the adsorbing particles become smaller; that is, as the "specific" surface of the particles becomes greater. If the particles are so small as to approach the dimensions of a molecule the adsorption process then becomes a chemical reaction. This apparently is the case with permutite. According to Schulze, at least, every molecule in permutite particles resides on the surface of the particle. Permutites are, therefore, adsorption combinations in stoichiometric proportions. This explanation clears up the controversy between Gans, Stremme, and others. In soils, such an evolution from adsorption to chemical combination is quite possible, especially with certain of the humus-substances. Ordinarily the particles are no smaller than colloids; most of them are larger even than  $0.1\mu$  as investigation has shown. The humus constituents occur in all degrees of fineness, and some of the particles are no larger than molecules.

The adsorbed bases play important parts in the processes taking place in the soil. It is therefore desirable to ascertain the amounts of these bases present in soils. The ordinary methods of soil examination are inadequate for the purpose.

It is apparent that any method for estimating the adsorbed bases depends on the properties of these bases towards other bases in the solution. Since the "exchanging" process is reversible the method chosen must be based upon a leaching process. Even on digestion with a moderately strong solution of ammonium chloride (Meijer method) all the adsorbed calcium does not go into solution. Now the question arises whether by leaching soils with solutions, for example, of ammonium, potassium, or sodium chloride, only the bases from the surface of the soil particles are removed or whether they are also removed from the interior of the particles in appreciable amounts. If this were the case then the exchange process in the soil would occur in this way: at first a fairly large amount of the base elements would go into solution, while with further leaching smaller quantities go into the solution. If only the adsorbed elements are dissolved the exchange process would, for all practical purposes, soon be complete. In order to study these questions, an exact investigation was conducted with clay soils and with sandy loams, all free from calcium carbonate. The experiment showed that a point was reached very quickly at which no more bases were given up to the solution. On leaching the soil with ammonium chloride, potassium chloride, or sodium chloride, therefore, only adsorbed bases go into solution.

As further ground for this conclusion, the speed of the exchange process was investigated. If only the adsorbed bases are replaceable equilibrium should be quickly reached. For the present only the exchangeability of calcium in a clay soil and in two sandy loams will be considered. For esti-

inating this capacity for exchange of bases, a weighed quantity of soil was treated with a definite amount of the chloride solution by shaking. Determinations were then made of the amount of calcium present in the solution after periods of 5 seconds, 1 minute, 1 hour, 1 day, 1 week. With the clay soil equilibrium was reached in less than 1 minute. With a sandy loam soil only 0.01 per cent calcium went into solution after 5 minutes. From consideration of these results it was seen that of the total quantity of calcium which could go into solution under these considerations in the clay soil, 97 per cent went into solution in the first 5 seconds, and for the sandy loams 90 per cent was in solution at the end of 5 minutes. Such results could only be obtained where the exchange process is confined to the easily accessible adsorbed bases.

The solubility of  $\text{CaCO}_3$  in water is low. In presence of  $\text{NaCl}$  and  $\text{KCl}$  it is not appreciably increased, but is increased to a considerable extent by  $\text{NH}_4\text{Cl}$  solution. On leaching soils containing calcium carbonate, with solutions of  $\text{NH}_4\text{Cl}$ ,  $\text{KCl}$ , and  $\text{NaCl}$ , more or less of the soil carbonate of lime, in addition to adsorbed calcium, is dissolved.

It was shown in an extensive investigation that the quantity of calcium carbonate which goes into solution on leaching the soil with  $\text{NH}_4\text{Cl}$  solution decreases as leaching proceeds. In the first liter of extract, therefore, more of the  $\text{CaCO}_3$  is dissolved than in the second. When using solutions of  $\text{KCl}$  or  $\text{NaCl}$  only small amounts of the carbonate dissolved. It was observed that with these two solutions this quantity is practically proportional to the quantity of the solution used for leaching (up to two liters). In the second liter there is practically as much  $\text{CaCO}_3$  dissolved as in the first. On leaching with  $\text{KCl}$  or  $\text{NaCl}$  solution the difference in the calcium content of the first and of the second liter represents the amount of replaced calcium from the soil.

It was found that in the soils examined no  $\text{MgCO}_3$  was present.

#### METHOD FOR ESTIMATING ADSORBED BASES

##### *1. Estimation of adsorbed lime and magnesia*

Twenty-five grams of soil (for mixed sandy loam soils 50 gm. may be used) were shaken in a beaker with 100 cc. of a warm normal solution of  $\text{NaCl}$ . This was occasionally shaken and allowed to stand over night. In this way the sample was thoroughly saturated with  $\text{NaCl}$  solution. The liquid was then poured through a filter into a liter flask, the mass of soil brought quantitatively on the filter and treated with successive portions of the solution. The filter was allowed to empty between each addition of  $\text{NaCl}$  solution. If the first portion of the filtrate was cloudy it was run through the filter again. When the flask was filled to the mark the funnel was placed in another liter flask and treatment continued until the second flask was also filled to the mark. The calcium content of the two filtrates was then determined. The difference in the calcium content of the first- and the second-liter portions corresponds to the replaced calcium. Adsorbed magnesium may be estimated in the same way although this is more conveniently determined as under (2).

The second liter contains only traces of magnesium; and in soils which do not contain calcium carbonate the second liter is practically free of calcium.

## 2. *Estimation of adsorbed magnesium, potassium, and sodium*

These elements were determined in a manner similar to (1) except that 25-gm. portions of the soil were leached out with successive portions of normal ammonium chloride and the washings were collected in two half-liter flasks.

## DISCUSSION

In this method the adsorbed bases are represented by the difference in quantities removed in the first and the second liter or half-liter portions. Even if further study should show that besides the adsorbed bases small quantities of that class of bases which I have designated as "acid-soluble" are also removed, the method would still permit the satisfactory determination of the adsorbed bases. The small quantities of acid-soluble bases removed would be proportional to the amount of leaching liquid. Thus if sodium chloride removed small quantities of acid-soluble calcium and magnesium the amounts removed in the first- and second-liter portions would be equal. Therefore the difference in calcium or magnesium content of the first and second liters would represent the exchangeable calcium or magnesium. The same considerations apply to ammonium chloride except that we deal with half-liter portions. From the same line of reasoning we need expect no serious difficulty due to impurities in the sodium chloride or ammonium chloride.

The adsorbed calcium was estimated in a large number of clay soils as described above, while for a part of these soils the adsorbed magnesium, potassium, and sodium was also estimated. The results are stated as the content of exchangeable bases, in percentages. From these percentages are calculated the content of exchangeable bases expressed in milligram-equivalents per 100 gm. of air-dry soil, and per hundred adsorbed ions. Thus, an average of the soils examined contained per 100 gm. of soil: 30.0 m.e. Ca, 5.0 m.e. Mg, 0.8 m.e. K, and 2.5 m.e. Na,—a total of 38.3 milligram-equivalents in 100 gm. of soil. Then in one hundred adsorbed cations there are 79 Ca, 13 Mg, 2 K, and 6 Na ions. The divalent ions predominate, calcium being the most prominent one.

Two sandy loam soils were examined for adsorbed bases. In the humus, there were 76.3 Ca ions, 13.1 Mg ions, 3.0 K ions, and 7.6 Na ions for each hundred adsorbed cations. In these soils the divalent ions also predominate.

The term "acid-soluble bases" designates the portion of Ca, Mg, K, and Na which goes into solution when the soil is treated with strong HCl, after deducting the adsorbed bases and the water-soluble chlorides, carbonates, etc. All clay soils examined have a low acid-soluble Ca and Na content (averaging 0.251 and 0.270 per cent) as compared with a high acid-soluble K and Mg content (1.340 and 0.826 per cent). Of the total calcium (adsorbed

and acid-soluble), 76.9 per cent was in the adsorbed condition. Of the total Mg, K, and Na contents only 5.6, 2.6, and 10.9 per cent respectively, were adsorbed. These bases occur for the greater part in the acid-soluble form, as contrasted with calcium, which is for the greater part adsorbed.

Two sandy loams were examined for acid-soluble bases. These differed from the clay soils in that the calcium ranked highest as the acid-soluble base. In the clay the greater part of the bases was in the acid-soluble form with the exception of calcium. With the sandy loams, the opposite relations were observed. For a fair comparison the *sum* of the total bases, should be compared with the sum of the adsorbed bases (both expressed in equivalents). Thus 100 gm. of a clay soil contained 137.7 m.e. of total bases and only 35.4 m.e. of adsorbed bases. Of the total bases (soluble in strong HCl) present in the clay soil about 25 per cent occurred as adsorbed bases. The sandy loam soils contained 36.9 m.e. of total bases, and 21.8 m.e. (or 59 per cent) of adsorbed bases. In the humus the major part of the bases occurred in the adsorbed form, while in the weathered mineral-complex (clay) the greater part occurred in the acid-soluble form. It is evident that the reason for this distinction is to be found in the variation in size of the particles in the mineral and in the organic weathered-complex. The smaller the particles the greater the ratio of surface to mass and naturally the greater the amount of adsorbed substances present.

The great importance of adsorbed bases in the soil processes is recognized both from the standpoint of clay soils and of sandy loams.

The amounts of adsorbed bases in milligram-equivalents designated as *S* varied in the clay soils examined from 23.3 to 48.9 (with the exception of a very low value of 12.4 for a soil B38) and with the loam soils examined, from 8.4 to 21.8. These values depend primarily on the content of adsorbing substance in the soil (clay-humus), and further, they decrease in the course of years due to action of plants and the percolating action of rain water.

In the moist climate of Holland the soils are adsorptively unsaturated.

The loss of a part of the adsorbed bases is partly caused by physiological agencies and partly by colloidal-chemical means. If a sufficient amount of adsorbed bases is lacking, the soil is in such a condition of unsaturation that the adsorbing soil complex can not efficiently function as the pH regulator. The soils then become acid. Besides, a change in the colloidal equilibrium of the soil occurs following such a desaturation of the soil, and this causes considerable modification of its physical nature (formation of hard-pans). With clay soils the effects of this desaturation on the colloidal-chemical processes are more noticeable. With loam soils the physiological effects are noticed to a greater or lesser extent. With these there is as much probability of a strongly alkaline as a strongly acid reaction in the soil solution. The range of optimum acidity of the soil solution, the acidity being expressed by the value pH, is more quickly changed in either direction with soils high in humus than is the case with clay soils. This difference may be due to the difference in strength of the clay- and the humic-acids.



The value of  $S$  (sum of adsorbed bases expressed as milligram-equivalents per 100 gm. of soil) gives us no positive insight into the real character of the soil. To fully understand this a knowledge of the degree of saturation of the soil is necessary. This term is used by the author to designate the ratio of the actual quantity of adsorbed bases in the soil  $S$  to the possible degree to which this might be extended; that is, to the amount of bases which the soil is capable of adsorbing  $T$ . A satisfactory procedure for the determination of  $T$  has not yet been worked out. A relation evidently exists between the value  $T$  and the clay-humus content, or,  $T$  varies with the content of clay humus substance. The values calculated on this assumption, which do not give the actual saturation capacities but are only proportional to them, were determined for a series of clays and for one sandy loam soil. With clay soils, the degree of saturation varied from 67 with the younger peat soils to 20 with older soils and "Katteklei"; in sandy loams it ran from 193 with calcareous valley soil to 64 with an old valley soil.

The colloidal chemical equilibrium depends not only upon the degree of saturation of the soil but also on the relation of the adsorbed bases one to another. For practical purposes, to these various relations of adsorbed cations may be ascribed that well known agricultural fact that calcic fertilizer materials act favorably on soil structure while sodium compounds have the opposite effect, and make the soil "sticky." Theoretically, the difference in effect of lime fertilization on the soil structure on the one hand, and the effect of fertilization with sodium compounds, on the other, is due to formation of gels in the first case and sols in the latter. Then the great variation in the deflocculating power of the di- and mono-valent cations on clay suspensions and humus fluids is due to the colloidal chemical effects of the adsorbed monovalent and divalent cations in the soil. Changes in these relations lead to changes in the equilibrium and influence changes in the soil structure.

The Rothamsted experiments have shown the unfortunate results of continued yearly applications of nitrate of soda on the soil structure. Likewise the ill effects of flooding fields with sea water is well known. Examination of such soils shows that they differ markedly from normal soils. As a rule they contain per 100 adsorbed cations only 56.9 Ca ions and 20.2 Mg. ions, or 77 divalent cations, against 23 monovalent cations. In normal soils the figures are 92 divalent to 8 monovalent cations.

Salts in sea water not only affect the adsorbing substances of mineral nature but also those of organic or humus nature. The effects are mainly on the calcium (gels) and sodium (sols) humates. The presence of the humus sols in soils treated with sea water is shown by extraction of such soils with water. Normal calcium-clay soils give bright yellow colored extracts; the extracts from the sodium-clay soils are yellow to brown in color.

It is evident that variations occur in the relations of adsorbed cations of water-borne soils according to whether the soil was deposited by fresh or salt water. In the latter instance, such as the so-called "Kwelder" soils, the soils

are distinguished by their high content of adsorbed monovalent cations (50 divalent and 50 monovalent, generally) while the fresh water action on soils leaves them with much smaller amounts of the monovalent cations. As the content of exchangeable calcium increases, the content of exchangeable sodium and of calcium carbonate decreases. One experiment seems to show that salt water clays always contain considerable exchangeable sodium. In this way soils which have been in contact with fresh water may be distinguished from those which have been in contact with salt water.

At the present it is generally assumed that plant nutrients are distributed through the soil in a slightly water-soluble form, so that neither a complete removal by leaching on the one hand or too strong a concentration of the soil solution on the other, can occur. But water, and especially carbonated water can always take up small quantities. As plants withdraw the dissolved materials from this dilute solution they are replaced to a greater or lesser extent by weathering of silicates or humus materials. It is worthy of note that this explanation of withdrawing of nutrients from the soil by the chemical changes taking place between adsorbing material of the soil and the substances dissolved in the soil water has not been elaborated before. The investigations of Prianischnikow have directed attention to these changes. From his investigations it was shown that potassium absorbed in potash-permutite could not be utilized by plants without the action of the other salts of the soil solution. The author has shown, moreover, that adsorbed potash is just as available for the plants' needs as the water-soluble potash. The same conclusion was also drawn as regards the ammonia of ammonium permutite. It does not matter if *only* the adsorbed bases are assimilated. Kellner has made a study of this and concludes that potash and calcium in the dissolved or adsorbed condition can serve as nutrients for legumes but cannot be taken up from difficultly-soluble compounds by the roots.

From evidence presented we cannot say that it is principally the adsorbed substances which are adsorbed. Perhaps the opinion of Adolph Meyer may well be considered, i.e., that the substances in the adsorbed condition are *not* the only ones available. But it seems that the adsorbed bases are of fundamental importance in absorption through the roots of plants. From this standpoint the determination of these bases deserves thorough attention.

In this connection it is well to give attention to the experiment and observations of Ehrenberg who has studied the "calcium-potassium-ratio." This ratio concerns to a great extent the great differences in content of adsorbed calcium and potassium in clay soils, and considers the general relation of the adsorbed bases with one another. "Kwelder" soils which are high in sodium and poor in calcium are spoken of as of the "calcium-sodium-type."

The question arises as to whether there is a reciprocal transfer between the adsorbed and acid-soluble bases. This cannot possibly occur to any great extent. If it were the case then we could not explain why the content of adsorbed and of acid-soluble bases in a soil had not become equalized after

existing so closely together for these hundreds of years. There should, for example, not be such great difference between the adsorbed magnesium (0.08 per cent) and potassium (0.024 per cent) and the acid-soluble magnesium (1.34 per cent) and potassium (0.826 per cent). Then also the lowering of the degree of saturation of soil, which actually occurs and to a considerable extent, could not be explained so long as these soils have such an excess of acid-soluble bases. From these considerations we must consider two forms of bases, the exchangeable or adsorbed bases, and the acid-soluble bases. Between these two classes no appreciable exchange occurs.

The influence of adsorbed bases on the soil processes, as previously mentioned is remarkable. The small amount of adsorbed potassium found in the soils examined, and averaging only 0.024 per cent is of greater importance for plant nutrition than the 0.826 per cent acid-soluble potassium. The degree of saturation of soils depends on the content of adsorbed bases. While this content in the course of years might decline only a little in comparison with the total-base content, yet even a small decrease has a great effect on the processes, physiological as well as colloidal-chemical, which take place in the soil. There is further the relation between the monovalent and the divalent cations which is also involved in governing the colloid-chemical equilibrium in the soil. In normal soils the adsorbed monovalent cations are outnumbered by the total number of adsorbed cations. The proportion of acid-soluble divalent cations to monovalent acid-soluble cations is not of any significance.

Even if it should be shown by future investigations that in my discussion the importance of adsorbed or exchangeable bases has been given too much emphasis and the acid-soluble bases undervalued, enough has been shown to indicate that the former are of greater importance to soil properties than the latter. At any rate the estimation of exchangeable or adsorbed bases should have a place in any extensive scheme of soil examination.

## NODULE-PRODUCTION KINSHIP BETWEEN THE SOY BEAN AND THE COWPEA

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### INTRODUCTION

There has been much speculation as to the relationship of the organisms from the nodules of the numerous species of legumes. Moore (13) has presented the extreme possibility of relationship between legume bacteria, namely, that only slight physiological differences exist between strains due to association with a certain legume species, which differences can be destroyed by cultivation upon artificially prepared media. This conclusion has not been borne out by later experimental work with improved methods.

### HISTORICAL

The data to be reported in this paper bear only on the varieties or strains of *B. radiculicola* Bey. isolated from soy bean *Soja max*, Piper, and cowpea *Vigna sinensis* (L.) Endl., and species related to the cowpea on account of the reported interchangeability of their nodule-forming organisms, for which reason the literature pertaining to groups other than these two will not be taken into consideration except when it has a close bearing on the subject.

There is a preponderance of evidence obtained by field observations and laboratory investigations to the effect that the bacteria which produce nodules on the soy bean will not produce them on the roots of any other common legume.

Kirchner (7) in observations made at Hohenheim, Germany, during a number of years failed to find nodules on the soy bean. Hiltner and Stormer (4), Stutzer (19) and Simon (18) confirm this condition. On the other hand, Kirchner (7, footnote) refers to Cohn's work at Breslau when he found nodules on the roots of plants of soy beans growing in soil to which no inoculating material of any kind had ever been applied.

As a result of pure culture studies Simon (18) concludes that *B. radiculicola* from soy bean does not cross inoculate with lupines and other legumes but his series of plants does not include the members of the cowpea group. This lack of relationship is in agreement with his serological work and that of Krüger (8).

The absence of the cowpea nodule-organism from certain soils in Austria is reported by Gross (3) and Simon (18) reports for German soil on which he experimented, the absence of nodule formation of *Lespedeza striata*, (Thunb.) H. and A., *Vigna sinensis*, and *Dolichos* species, all of which belong to the cowpea group.

In the United States, it has been observed over a period of years at experiment stations and on farms that it is usually necessary to introduce soy bean bacteria when soy beans are being planted for the first time. Otis (14) found that the soy bean organism was lacking in the soils at the Manhattan station yet Hitchcock (5) reports at about the same time, the presence of *Meibomia* sp., *Lespedeza* sp., and nodule-producing *Cassia* sp. growing in the vicinity of the station. Lewis and Nicholson (9) found that the cowpea organisms were abundant in the soils of the experiment station farm whereas soy bean plants did not have nodules on their roots. Lipman (10) reports that at Hammononton, N. J., without the introduction of inoculation soy beans produced no nodules whatever although cowpeas had numerous nodules on their roots. Hopkins (6) makes a similar report. Perhaps the literature of other experiment stations might tend to make the case more complete but sufficient of the field observations have been given to show the unanimity of the results.

These field observations may be supplemented by the pure-culture work of Garman and Didlake (2) from which they conclude that the soy bean nodule bacteria are a distinct species, not forming nodules on cowpea, garden bean or garden pea. Burrill and Hansen (1) reach practically the same point of view as the result of vegetative tests but find between the cowpea and soy bean organisms certain similar characteristics such as rate of growth, color and consistency.

Morphological studies by Löhnis and Hansen (12) and by Shunk independently (17) have shown that the soy bean and cowpea nodule bacteria possess polar flagella in contradistinction to the peritrichous flagella of cultures of *B. radicola* from other legumes shown to be unrelated to these two by vegetative test. Another similarity has been brought out by Löhnis and Hansen (12) namely, the effect of the peritrichic and monotrichic bacteria on sterile milk. The former cause a clear zone to form on the top of the milk in tubes but the latter in a reasonable length of time do not apparently alter the milk.

#### METHODS

##### *Isolation of cultures*

In view of the extreme variance of the data to be submitted with that already on record it has been deemed advisable to give somewhat in detail the methods employed in securing these results although many of the processes are common and quite well known.

Nodules selected for inoculum were washed, sterilized for about five minutes in mercuric chloride solution made up at the rate of 1 gm. of chloride, 500 cc. water, and 2.5 cc. HCl. The nodules were then washed with five different lots of sterile distilled water to eliminate the mercury salt. The above method was used only on nodules from which cultures were isolated previous to five years ago, subsequent isolations were made from nodules which were first thoroughly washed in tap water and then five times in sterile distilled water. Sterile conditions were maintained throughout the isolation by flaming the nodules using a sterilized scalpel to cut open the nodule for removing a small amount of inoculum from its interior. This inoculum was placed in sterile distilled water and shaken thoroughly following which it was further diluted, and transferred to sterile Petri dishes by sterile pipettes. Previous to five years ago Moore's synthetic nitrogen-free agar (13) was used in this work. Since then, however, and at the present time Löhnis soil extract mannite agar (11) has been employed. Plates were incubated in an insulated chamber

usually at living room temperatures ranging from 20° to 27°C. Characteristic colonies were selected and transferred to agar slants. The number of times a culture has been plated is dependent on the use to which it has been put. Some of the older cultures used in this work have been plated as high as twenty times but the usual number of platings has been but two or three; usually after the second plating the stock was multiplied in agar and broth for carrying on vegetative tests.

#### *Vegetative tests*

Building sand was washed in running water for one day, drained and placed on a clean table to air-dry. After drying in air the sand was put into a drying oven for a day or until thoroughly dry after which it was sieved to secure a uniform product. This sand was mixed with a small percentage of calcium carbonate and put in 32-ounce wide-mouth flint-glass bottles, 412 gm. per bottle. The sand was then moistened with 100 cc. of a nutrient solution made from a formula modified from Sachs (16) as follows:

Tap water.....	10,000 cc.
Calcium monophosphate.....	0.5 gm.
Sodium chloride.....	2.5 gm.
Potassium chloride.....	2.5 gm.
Magnesium sulfate.....	5.0 gm.

These bottles were closed with absorbent cotton plugs and sterilized for one hour at 17 pounds steam pressure.

It is practically impossible to obtain seed, especially the larger sized seed, which are free from foreign organisms but it is not a difficult matter to sterilize such seed to eliminate the legume organism. As a matter of fact, I have found that very few commercial leguminous seed carry any legume bacteria on or in their seed coats. The process of sterilization which was used in this work included both the hydrogen peroxide method of Robinson (15) and the mercuric chloride method previously mentioned in connection with the sterilization of nodules. The time of exposure with the latter disinfectant was doubled when seed were sterilized.

Seeds which had been sterilized were introduced into the sterile sand in bottles by a sterile platinum spoon in the protection of a culture room. These seeds were covered by agitating the sand with blows of the hands on the outside of the bottle. When growth was first apparent the culture to be tested was added in the culture room by means of a sterile pipette after which the bottles were transferred to the greenhouse in order to give the inclosed plants the light necessary for best growth. The cotton plugs were covered with paper to prevent evaporation and keep dust and other foreign substances from settling on them. Since the plants only grew a few months, it was unnecessary to add water. Water transpired by the plant was condensed when it came in contact with the shoulder of the bottle and thus was carried back to the sand to be used again by the roots of the plant. It is recognized that the plants in such bottles

as these are under very abnormal conditions, especially as regards the process of photosynthesis and respiration but there occurred sufficient growth of root and shoot in the short period of experiment to allow for the development of nodules. Each series of bottles was checked by controls which were untreated with the legume organisms and in no case have nodules been found on the roots of plants from these bottles. This, it is assumed, is sufficient evidence to warrant the statement that the seed planted were sterile with reference to *B. radiculicola*. Each test was at least made in quintuplicate.

#### OBSERVATIONS

In an endeavor to group the nodule organisms of Adzuki bean, *Phaseolus angularis* Willd. in 1917 under pure culture conditions it was observed that nodules were produced on Adzuki bean roots by cultures of *B. radiculicola* from soy bean, cowpea and Japan clover, and that a culture of nodule bacteria from Adzuki bean not only caused nodules to form on cowpea but also on soy bean. Other experimental work demonstrated similar relations between certain cowpea and soy bean cultures which led to the belief that there might be an unrecognized kinship between these organisms.

Further experimentation gave results which strengthened the case very much. The facts as gathered are presented in table 1.

#### DISCUSSION

The nodules formed by soy bean cultures on cowpea roots have been observed to be as normal in appearance and structure as those produced under similar conditions on the natural host. On the other hand, in a few instances nodules on soy bean roots produced by cowpea bacteria were more disk shaped than the normal nodules which were spheroidal in shape.

The facts presented in the foregoing table indicate a physiological relationship between the root nodule organisms from cowpea and related species and those of the soy bean. It will be noted that in every instance the soy bean organisms produced nodules on a variety of the normal host species as well as on the roots of a variety of cowpea. Cowpea bacteria, however, have given rather inconsistent results, characterized by numerous failures to produce nodules on the roots of soy bean plants.

The question which arises first is one which concerns the purity of the cultures. In view of the numerous purifications by plating of most of the soy bean cultures without altering their ability to function on the roots of both legumes, and the consistency with which the cross takes place with cultures of varying ages and from various sources, it seems almost an absurdity to consider that the results may be due to mixed cultures. If they are mixed they were probably in this condition in the nodule and are inseparable by any means known to the bacteriologist of the present time. The condition as found leads to the belief that the root hairs of the cowpea are more penetrable by the soy bean nodule organism than are those of soy bean by the cowpea organism.

TABLE 1  
Evidence of a cross inoculation relationship between the cowpea and soy bean strains of *B. radiculicola*

CULTURE NUMBER	SOURCE OF <i>B. RADICULICOLA</i>	AGE	NODULE PRODUCTION ON			
			Barchet soybean		Brabham cowpea	
			Total nodules	Average per plant	Total nodules	Average per plant
		years				
Control			0 - 54*	0	0 - 13*	0
179	Lima Bean.....	9.25	0 - 25	0	31 - 13	3-
182	Brabham cowpea.....	9.25	1 - 13	0	47 - 14	3+
185	Cowpea variety.....	9.25	0 - 20	0	30 - 12	3-
213	Adzuki bean.....	7.75	9 - 19	1-	18 - 9	2
216	Groit cowpea.....	7.75	7 - 14	1-	35 - 14	2+
220	Beggarweed.....	7.75	0 - 22	0	41 - 12	3+
243	Lima bean.....	7.75	0 - 25	0	27 - 14	2-
277	Groit cowpea.....	6.75	0 - 16	0	56 - 14	4
278	New Era cowpea.....	6.75	10 - 15	1-	49 - 15	3+
294	Japan clover.....	5.75	0 - 19	0	37 - 12	3+
300	Tepary bean.....	6.75	17 - 20	1	42 - 7	6
309	New Era cowpea.....	5.75	0 - 23	0	26 - 12	2+
313	Furze.....	4.75	73 - 16	5-	26 - 18	1+
316	Moth bean.....	4.75	1 - 23	0	18 - 9	2
321	Adzuki bean.....	4.75	19 - 15	1+	33 - 11	3
322	Velvet bean.....	3.75	0 - 21	0	47 - 10	5-
323	Velvet bean.....	3.75	0 - 18	0	24 - 12	2
324	Moth bean.....	3.75	23 - 19	1+	33 - 13	3-
325	Mung bean.....	3.75	0 - 22	0	29 - 13	2+
326	Brabham cowpea.....	3.75	9 - 10	1-	37 - 15	2+
333	Pigeon pea.....	2.75	0 - 21	0	8 - 3	3-
348	Tepary bean.....	2.75	3 - 12	1-	18 - 16	1+
349	Brabham cowpea.....	2.75	0 - 26	0	45 - 13	3+
351	<i>Acacia melanoxylon</i> .....	2.75	0 - 18	0	29 - 15	2-
354	Lyon bean.....	2.75	0 - 18	0	30 - 12	2+
369	<i>Cassia nitilans</i> .....	2.75	56 - 20	3-	41 - 12	3+
376†	Cowpea variety.....	?	29 - 29	1	61 - 24	3-
432†	Cowpea variety.....	?	2 - 14	1-	22 - 3	7+
434‡	Cowpea variety.....	?	18 - 25	1-	10 - 4	2+
180	Soy bean variety.....	9.25	26 - 12	2+	15 - 13	1+
181	Roosevelt soy bean.....	9.25	74 - 18	4+	27 - 12	2+
187	Roosevelt soy bean.....	9	48 - 24	2	29 - 16	2-

\* Total number of nodules is given by first figure and number of plants by the figure following the dash.

† Culture furnished by Dr. A. L. Whiting, University of Ill. Urbana, Ill.

‡ Cultures furnished by Dr. W. A. Albrecht, University of Missouri, Columbia, Mo.

§ Cultures furnished by Laboratory of Plant Physiology, N. Y. State College of Agriculture, Ithaca, N. Y.



TABLE 1—Continued

CULTURE NUMBER	COURSE OF <i>B. RADICICOLA</i>	AGE	NODULE PRODUCTION ON			
			Barchet soybean		Brabham cowpea	
			Total nodules	Average per plant	Total nodules	Average per plant
		<i>years</i>				
218	Barchet soy bean . . . . .	7.75	65 —17	4—	12 —14	1—
255	Virginia soy bean . . . . .	6.75	40 —10	4	15 —8	2—
256	Taha soy bean . . . . .	6.75	50 —28	2—	24 —10	2+
258	Tokio soy bean . . . . .	6.75	116 —31	4—	23 —9	3—
270	Cloud soy bean . . . . .	6.75	12 —7	2—	31 —15	2+
272	Arlington soy bean . . . . .	6.75	55 —21	3—	27 —18	2—
273	Hope soy bean . . . . .	6.75	40 —19	2+	22 —15	2—
275	Pingsu soy bean . . . . .	6.75	33 —20	1+	27 —10	3—
289	Chinese soy bean . . . . .	6	47 —20	2+	21 —8	3—
293	Barchet soy bean . . . . .	5.75	51 —20	3—	18 —11	2—
310	Barchet soy bean . . . . .	5.75	41 —14	3—	16 —11	1+
311	Chinese soy bean . . . . .	4.75	51 —18	3—	17 —14	1+
328	Barchet soy bean . . . . .	3.75	41 —13	3+	16 —8	2
329	Chinese soy bean . . . . .	3.75	42 —17	2+	13 —13	1
334	Haberlandt soy bean . . . . .	2.75	49 —20	2+	18 —15	1+
335	Chinese soy bean . . . . .	2.75	55 —21	3—	15 —11	1+
336	Virginia soy bean . . . . .	2.75	73 —18	4+	20 —10	?
399	Wilson soy bean . . . . .	1.25	46 —24	2+	22 —17	1+
400	Wilson soy bean . . . . .	1.25	48 —22	2+	26 —10	3—
403	Guelph soy bean . . . . .	0.75	52 —23	2+	15 —9	2—
429 <sup>  </sup>	Soy bean variety . . . . .	6	66 —19	3+	90 —15	6
430 <sup>¶</sup>	Manchu soy bean . . . . .	?	7 —7	1	2 —2	1
431 <sup>†</sup>	Soy bean variety . . . . .	?	13 —19	1—	20 —16	1+
433 <sup>‡</sup>	Soy bean variety . . . . .	?	45 —17	2+	14 —10	1+

¶ Culture furnished by Dr. E. B. Fred, University of Wisconsin, Madison, Wisconsin.

|| Culture of Prof. Roy Hansen furnished by Dr. F. Löhnis, U. S. Dept. of Agriculture.

Indeed the data presented and the results of field observations by other investigators gives the impression that the relationship is quite one sided. Work is in progress to determine whether bacteria from nodules produced on cowpea plants by soy bean bacteria possess more tendency to infect the root hairs of the soy bean than cultures of cowpea organisms from normally produced nodules, and other questions concerning this subject which have arisen during the course of this preliminary study.

#### SUMMARY

An interchangeability of the nodule-forming function between cultures of *B. radicicola* from soy bean *Soja max* and cowpea *Vigna sinensis* is indicated by work carried out under pure culture conditions.

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## THE RELATION OF SEED WEIGHT TO THE GROWTH OF BUCKWHEAT IN CULTURE SOLUTION<sup>1</sup>

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During the past year a series of experiments dealing with the relation of seed weight to germination, subsequent growth of the plants, and crop production was begun. It is the purpose of this paper to report briefly and in a preliminary way the results of a portion of this work which had to do with the growth of buckwheat plants (*Fagopyrum esculentum* Moench.) in culture solutions under experimental conditions, with respect to the environmental complex, which was approximately the same for all the plants.

It has long been recognized, of course, that the early growth and the subsequent development of plants may be greatly influenced by the amount and quality of the food materials stored in the seed. However, the general principles underlying and governing this relation are not at all well understood.

It is not the purpose here to consider uniformity in the weight of seeds in relation to the degree of variability of the plants grown from them but merely to study, from the standpoint of several quantitative plant measurements, the growth rates of the plants as these are influenced by the weight of the seeds from which they are grown. It is interesting to note, however, that the results obtained do indicate very definitely that plants grown from seeds selected for uniformity in weight show markedly less variability than do similar plants grown from seeds not so carefully selected.

It is a pleasure here to acknowledge grateful thanks to Dr. J. W. Shive for valuable suggestions, criticism, and aid in planning and executing the work, and to the various members of the department staff for helpful and timely aid during the progress of the investigation.

### METHODS OF PROCEDURE

Seeds of a commercial strain of Japanese buckwheat were weighed accurately to the tenth of a milligram. Of these, five weight-grades of seeds were selected as follows: (1) 40.5 mgm., (2) 35.5 mgm., (3) 32.5 mgm., (4) 29.5 mgm., and (5) 23.5 mgm. No seed was used which varied more than 0.4 mgm. from the weight grade value. The seed selected for each particular grade were germinated in grade-groups on a germinating net as described

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by Shive (4) and from the relatively large number of seeds germinated in each group five seedlings selected for uniformity of size and vigor were chosen for the experiment.

When the cotyledons had fully opened the seedlings were transferred to the culture vessels which consisted of bottles having a capacity of approximately 1050 cc. The neck of each bottle which was about 4 cm. in diameter, inside measurement, was file-marked. Each bottle was fitted with a paraffined cork stopper and marked with the number of the plant assigned to it and with the seed-weight grade from which the seedling was selected. Each bottle with the cork stopper was carefully weighed empty and again when filled to the file-mark with solution at a temperature of 21°C., all such weights being taken carefully to the tenth of a gram on a torsion balance.

For the early physiological growth period extending from germination to the flowering stage, Shive's three-salt solution  $R_3C_3$  was used. This solution has been found well adapted for the growth of buckwheat (5) and contained the three salts  $KH_2PO_4$ ,  $Ca(NO_3)_2$ , and  $MgSO_4$  in concentrations of 0.0144 m., 0.0052 m., and 0.0200 m., respectively. To each liter of solution used, 0.5 mgm. of iron in the form of a freshly prepared solution of ferrous sulfate was added.

After the seedlings were transferred to the culture solutions the cultures were placed on a rotating table in order to insure to all the plants similar environmental conditions. At the end of each 3.5-day interval throughout the growth period the cultures were removed from the rotating table, the old solution discarded, and the bottles again filled to the file-mark with new solution at 21°C. The cultures were then weighed and returned to the rotating table.

At the end of the seventh growth interval when the plants were in full bloom, Shive's three-salt solution  $R_3C_3$  was substituted for that used during the early growth stage. This change is necessary, according to Shive and Martin (6), in order to produce optimum growth of buckwheat plants in these three-salt solutions during the period from the flowering stage to maturity.

The plants were harvested after they had been in culture 42 days and the dry weight of tops and roots and the leaf area of each plant were obtained separately. The leaf areas were secured by blue-printing the fresh leaves and subsequently determining the areas of these leaf-prints for each plant by means of the planimeter.

An attempt was here made to obtain the total green weight (tops and roots) of each plant at the end of the various growth intervals throughout the entire growth period. At the end of the growth intervals each plant was removed from its culture bottle together with the cork stopper in which it was mounted, placed in an empty container and allowed to drain thoroughly. The old solution was then discarded and the culture bottle filled to the file-mark with new solution at 21°C., the last few cubic centimeters of solution being added from a burette for the sake of greater accuracy. The plants were then replaced and each culture accurately weighed to the nearest tenth of a gram on a torsion balance. By deducting the weight of the filled bottle and cork stopper from the total weight the approximate green weight of the plant as well as the increase in weight from interval to interval was derived. The method is faulty in some respects. It does not take into account the weight of the solution adhering to the roots after draining, but since the error thus introduced is approximately the same for all the plants the relations indicated by the green weight data thus obtained should not be appreciably different from the true relations. Its accuracy is also decreased by variations due to transpirational fluctuations of the individual plants.

#### EXPERIMENTAL DATA

##### *Green weights*

In table 1 are given the total green weights of the plants as these were here derived. The first green weights were taken on the tenth day after the plants

were placed in the culture solution and at the end of each succeeding 3.5-day growth interval. The weights of the five plants and the averages of these are shown for each seed-weight grade.

TABLE 1  
*Green weights of plants obtained at intervals throughout the growth period*

SEED-WEIGHT GRADE	WEIGHTS AFTER VARYING PERIODS OF GROWTH IN CULTURE									
	10.0 days	13.5 days	17.0 days	20.5 days	24.0 days	27.5 days	31.0 days	34.5 days	38.0 days	41.5 days
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
40.5 mgm.....	4.0	6.0	8.0	8.6	13.0	15.5	15.0	16.5	20.0	18.2
	5.0	6.7	9.7	10.6	16.6	19.8	20.9	24.4	24.9	25.4
	5.0	5.8	7.5	7.5	12.5	14.3	15.2	16.5	17.5	16.5
	3.7	6.2	6.1	8.1	9.8	12.1	12.6	14.5	14.6	15.1
	3.0	5.5	6.8	8.6	10.6	11.6	13.4	13.6	15.6	16.0
Average.....	4.14	6.04	7.6	8.7	12.5	14.5	15.4	17.1	18.5	18.2
35.5 mgm.....	4.0	6.2	8.0	8.0	12.0	16.0	17.0	17.4	19.6	20.8
	4.6	9.1	8.2	10.6	13.1	15.6	15.8	16.6	16.8	18.3
	4.4	7.3	8.4	9.6	11.6	15.1	16.4	17.6	18.1	20.4
	4.2	7.0	8.5	10.5	14.5	17.0	17.5	20.7	21.7	23.0
	3.8	6.3	8.3	10.1	13.1	15.3	14.9	16.6	17.6	18.6
Average.....	4.2	7.18	8.3	9.76	12.86	15.8	16.3	17.8	18.76	20.26
32.5 mgm.....	4.2	7.0	8.0	8.7	12.7	14.2	14.7	15.9	16.4	17.2
	5.0	6.2	6.6	7.8	12.1	14.6	16.6	18.6	19.3	20.1
	3.0	5.4	7.0	6.2	10.7	14.4	14.2	15.7	17.2	17.6
	3.2	5.0	6.7	6.9	10.4	11.9	13.7	14.7	15.9	15.4
	4.6	6.9	8.1	10.5	14.9	16.9	16.9	18.4	19.7	18.9
Average.....	4.0	6.1	7.3	8.02	12.16	14.4	15.2	16.66	17.7	17.84
29.5 mgm.....	5.3	6.8	9.3	10.3	13.4	16.1	18.1	18.8	20.3	19.8
	3.1	5.6	6.8	6.8	11.8	13.8	15.0	17.8	18.2	17.8
	3.0	5.5	7.2	7.7	11.3	11.9	13.7	14.7	15.3	14.7
	4.3	5.8	7.3	8.8	12.5	14.3	15.8	17.8	18.3	17.8
	3.5	6.5	8.3	8.8	12.3	14.8	15.8	16.8	16.3	17.8
Average.....	3.94	6.04	7.8	8.7	12.26	14.2	15.7	17.2	17.7	17.58
23.5 mgm.....	3.5	5.2	6.5	6.8	10.3	12.3	14.3	15.4	16.8	17.3
	2.7	5.5	6.3	6.3	10.2	12.8	13.3	14.8	15.6	16.8
	3.0	5.4	6.8	8.4	12.9	14.1	16.3	17.1	17.1	17.7
	3.0	3.5	6.8	6.9	10.1	11.4	13.4	14.0	14.9	13.4
	4.2	6.8	8.2	9.5	13.3	15.5	16.5	18.8	21.5	21.3
Average.....	3.7	5.3	6.9	7.6	11.36	13.3	14.76	16.04	17.2	17.3

From the data of table 1 it will be observed that the green weight averages for the plants grown from the smallest seeds (23.5 mgm. grade) are the lowest throughout, while those from the next to the largest seeds (35.5 mgm. grade) are the highest throughout and that the order of superiority of the plants grown

from seeds of different weight corresponds to the order of seed weight from the lowest to the next to the highest here used. The plants grown from the abnormally large seeds (40.5 mgm. grade) were slightly inferior in average green weight throughout to those grown from the large medium seeds (35.5 mgm. grade).

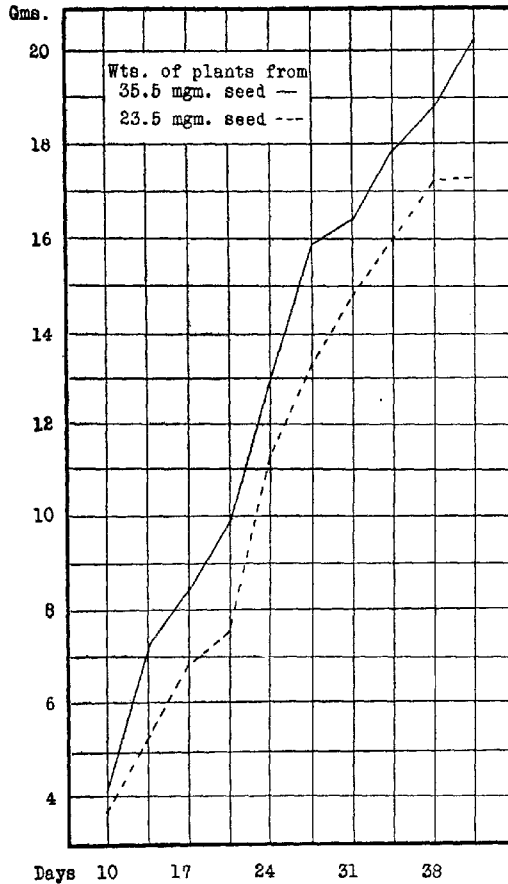


FIG. 1. GRAPHS OF AVERAGE GREEN WEIGHTS OF PLANTS GROWN IN SOLUTION CULTURES FROM SEEDS WHICH PRODUCED THE HIGHEST AND LOWEST YIELDS

The average green weight data for the plants grown from the grades of seeds which produced the highest and the lowest yields are plotted to form the graphs of figure 1. The graphs representing the average green weight data corre-

sponding to the remaining three seed-weight grades are here omitted since they occupy positions intermediate between the two graphs shown in figure 1, and do not intersect them at any point.

TABLE 2  
*Dry weights and leaf areas of plants grown in culture solutions*

SEED-WEIGHT GRADE	DRY WEIGHTS			LEAF AREAS
	Tops	Roots	Total	
	gm.	gm.	gm.	sq. cm.
40.5 mgm.....	1.6615	0.1061	1.7676	223.1
	2.0950	0.1677	2.2627	312.2
	1.5546	0.0918	1.6463	225.8
	1.2300	0.0728	1.3028	202.1
	1.1257	0.1151	1.2408	181.1
Average.....	1.5333	0.1107	1.6440	228.9
35.5 mgm.....	1.4615	0.1344	1.5959	241.4
	1.6035	0.1067	1.7092	235.8
	1.6004	0.1205	1.7209	241.4
	1.7793	0.1040	1.8833	221.7
	1.5026	0.1547	1.6573	254.9
Average.....	1.5895	0.1239	1.7133	239.0
32.5 mgm.....	1.4425	0.0617	1.5042	212.8
	1.4842	0.1250	1.6092	226.9
	1.6840	0.1271	1.8111	243.6
	1.3522	0.1224	1.4746	221.2
	1.7814	0.1066	1.8880	245.0
Average.....	1.5489	0.1086	1.6574	229.9
29.5 mgm.....	1.5057	0.1327	1.6384	231.8
	1.6638	0.1164	1.7802	236.9
	1.4952	0.1208	1.6160	231.5
	1.4652	0.1294	1.5946	227.0
	1.2745	0.1175	1.3920	170.7
Average.....	1.4809	0.1234	1.6042	219.6
23.5 mgm.....	1.3587	0.0946	1.4533	202.6
	1.2040	0.0982	1.3022	182.6
	1.3590	0.1267	1.4857	195.3
	0.9260	0.0909	1.0169	147.5
	1.7540	0.1561	1.9101	272.9
Average.....	1.3163	0.1133	1.4336	200.2

It is clearly apparent from the graphs of figure 1 that the advantage, with respect to green weights, in favor of the plants grown from the heavier seeds over those from the lighter seeds is not only maintained throughout the entire growth period but is also gradually, though not very greatly, augmented as the plants become older. The average green weights of the plants grown from



the heavier seeds were, at the end of the first and last growth intervals represented on the graphs, 13.1 per cent and 17.1 per cent heavier, respectively, than were the corresponding weights of the plants grown from the lightest seeds employed.

*Dry weights and leaf areas*

The absolute dry weight yields and the leaf areas of the mature plants together with the averages of these plant measurements corresponding to each seed-weight grade are presented in table 2. The average dry weight yields of

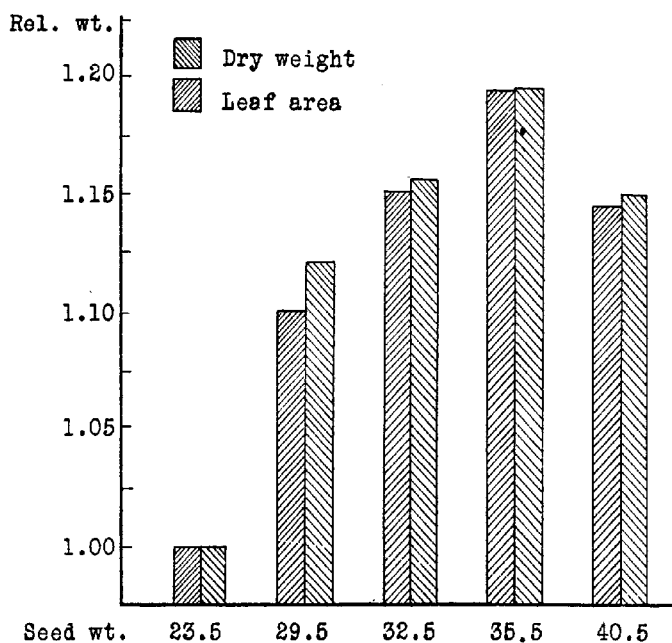


FIG. 2. DIAGRAM COMPARING AVERAGE RELATIVE DRY WEIGHTS WITH AVERAGE RELATIVE LEAF AREAS OF PLANTS GROWN FROM SEEDS OF DIFFERENT WEIGHTS

tops and the average leaf areas are shown diagrammatically in comparison in figure 2, but all the values are here expressed in terms of those corresponding to the lowest seed-weight grade (23.5 mgm.) taken as 1.00.

As is clearly brought out by the diagram of figure 2, the order of superiority of the plants grown from the seeds of different weights, with respect to average dry weights of tops, average total dry weights, and average leaf areas, corresponds to the order of seed weights from the lowest to the next to the highest,

this relation being, therefore, in absolute agreement with that of the green weight values. No such relation, however, exists between seed weights and dry weights of roots, although the seed-weight grade corresponding to the highest average yield of tops, total yield, and leaf area corresponds also to the highest dry weight of roots. The average weight of tops, total dry weight, and leaf area obtained with the seeds from the highest yielding weight-grade (35.5 mgm.) are 20.8 per cent, 19.5 per cent, and 19.4 per cent higher, respectively, than are the corresponding yield values obtained with the seeds from the lowest weight-grade (23.5 mgm.).

It is interesting and important to note that the average leaf areas are nearly proportional to the average dry-weight yields of tops, as is apparent from the diagram of figure 2 and from the data of table 2. This is in entire accord with the work of McLean (3) and Hildebrandt (1, 2) who found that the leaf areas of soybean plants at the age of four weeks are approximately proportional to the dry weight of stems and leaves. Since, as is clear from the data of table 2, the total dry weights of the buckwheat plants here used are approximately proportional to the dry weights of tops, this relation holds also between total dry weight yields (tops and roots) and leaf areas. On the other hand, no such relation is apparent between dry weights of roots and leaf areas. This follows, of course, since there is no definite correlation between the growth of tops and roots of the buckwheat plants here employed.

It is to be emphasized, of course, that the data here presented are too meager and inadequate to justify anything more than suggestion. Definite conclusions are not warranted and the purpose of the paper has been achieved by a brief consideration of some of the more important points which the data have conveyed.

#### SUMMARY

Buckwheat plants were grown in solution cultures from seeds of five different weight-grades, under experimental conditions which were approximately alike for all the plants.

1. Seeds of high medium weight produced better plants, from the standpoint of several quantitative plant measurements (averages only considered), than did seeds of lighter weight or abnormally heavy seeds.
2. The order of superiority of the plants grown from the heavier seeds over those grown from seeds of lighter weight corresponds to the order of seed-weight, except for the abnormally heavy seeds.
3. Under conditions which were approximately the same for all plants, the superiority of those grown from heavier seeds over those grown from lighter seeds was maintained from the early seedling phase to maturity.
4. Leaf areas were approximately proportional to dry weights of tops and to total dry weights but no such relation was apparent between dry weights of roots and leaf areas.

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## SOLUBILITY OF LIMESTONES AS RELATED TO THEIR PHYSICAL PROPERTIES

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### INTRODUCTION

Limestone varies widely in mineral structure and chemical composition. Its extensive use in agriculture in the correction of soil acidity creates a need for some accurate basis for the establishment of its value for this purpose. The total neutralizing power is commonly accepted as an absolute measure of agricultural value. From an economic standpoint however, the farmer is primarily interested in the rate of reaction of the limestone, since upon this factor depends the rapidity with which the undesirable conditions due to acidity will be corrected.

The effects of fineness of grinding and of chemical composition upon the rate of solubility of limestone have been studied in detail by several workers, notable Frear (5), Ames and Schollenberger (1), Barker and Collison (2), and Stewart and Wyatt (9). No experimental data has yet been published showing the influence of physical properties such as porosity, hardness, specific gravity and crystalline structure upon the rate of reaction.

Frear (4, p. 175) states that it is entirely probable that a porous, impure limestone will break down more rapidly than a compact, pure stone because such is the case with massive rocks showing similar differences in texture and purity. In a later publication (5) he adds that the increased solubility of porous stone may not be as great as otherwise expected, due to the relatively much smaller solubility in the capillary pores than on the free surface.

Lacking definite information upon this subject, popular opinion has been that the crushed or ground product from soft, porous limestones is more rapidly soluble than the material from hard, compact rocks of similar chemical composition. In an effort to throw some light upon this subject, the authors have investigated the solubility of a series of limestones showing marked differences in their physical properties.

### EXPERIMENTAL

A series of twelve limestone materials, varying widely in their physical and chemical nature and described in tables 1, 2 and 3 were selected for this study. Solubility was determined by measuring the rate of reaction with dilute standard acetic acid (table 4), the decrease in lime requirement of acid soils produced

TABLE I  
*Descriptive data on limestones*

NUMBER	SOURCE	FORMATION	HARDNESS	POROSITY	STRUCTURE	COLOR	COMPOSITION
1	Martinsburg, W. Va.	Stone's River	Very hard	Very compact	Very fine grained non-crystalline	Dove	High-calcium moderate purity
2	Muskingum Co., Ohio	Maxville	Very hard	Compact	Fine grained non-crystalline	Blue-gray	High calcium
3	Fremont, Ohio	Monroe	Medium	Medium	Medium grained non-crystalline	Drab	Dolomite moderate purity
4	Youngstown, Ohio	Putnam Hill	Very hard	Compact	Coarse grained non-crystalline	Dark blue-gray	High calcium slightly impure
5	Piqua, Ohio	Brassfield	Medium	Medium	Coarsely crystalline	Light gray	Slightly dolomitic
6	Piqua, Ohio	Brassfield	Medium soft	Medium	Very crystalline	White	Moderately dolomitic
7	Kelly Island, Ohio	Columbus	Medium soft	Medium	Coarse grained non-crystalline	Gray	Moderately dolomitic
8	Port-of-Calcite, Mich.	Dundee	Soft	Porous	Medium grained non-crystalline	Light gray	Very pure high calcium
9	Carey, Ohio	Niagara	Medium hard	Very porous	Very crystalline	White	Pure dolomitic
10	Yellow Springs, Ohio	Springfield	Hard	Very porous	Coarse, partly crystalline	Rusty gray	Dolomite
11	Yellow Springs, Ohio	Travertine	Very soft	Very porous	Coarse, loose non-crystalline	Yellowish gray	High calcium
12	New Hampshire	Marble	Soft	Very compact	Medium grained very crystalline	White	High calcium

by the several materials at the end of varying periods of time (table 5) and the diminution of the carbonate content produced during a given period of exposure to acid soil conditions (table 6).

TABLE 2  
*Results of physical tests*

NUMBER	PORE-SPACE IN TOTAL VOLUME	SPECIFIC GRAVITY	HYGROSCOPIC CAPACITY		ABRASION IN 2 HOURS
			50 mesh	100 mesh	
	<i>per cent</i>		<i>per cent</i>	<i>per cent</i>	<i>per cent of initial weight</i>
1	0.16	2.717	0.098	0.110	6.00
2	1.46	2.751	0.454	0.456	6.25
3	9.23	2.823	0.138	0.175	7.10
4	0.48	2.718	0.200	0.250	5.65
5	4.43	2.759	0.195	0.185	7.90
6	0.49	2.756	0.054	0.060	9.15
7	14.10	2.769	0.062	0.078	11.25
8	13.40	2.653	0.070	0.087	11.65
9	18.21	2.781	0.185	0.188	8.30
10	17.62	2.790	0.216	0.269	7.60
11	45.00	2.465	0.440	0.458	39.45
12	0.39	2.723	0.042	0.060	28.20

TABLE 3  
*Chemical analyses*

NUMBER	CaCO <sub>3</sub>	MgCO <sub>3</sub>	SiO <sub>2</sub>	Fe <sub>2</sub> O <sub>3</sub>	OTHER OXIDES	NEUTRALIZING POWER CaCO <sub>3</sub> =100
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	
1	86.30	1.74	9.81	1.41	1.68	88.37
2	89.80	1.98	3.86	1.44	1.15	92.16
3	46.70	38.86	8.06	0.59	5.91	92.91
4	76.20	1.32	14.42	2.99	3.71	77.77
5	81.30	7.68	5.42	1.92	1.70	90.45
6	82.80	12.39	2.91	0.86	0.55	97.55
7	79.60	13.85	4.13	1.01	1.52	96.10
8	96.20	1.17	0.86	0.38	0.14	97.60
9	54.10	44.00	0.74	0.36	0.38	106.50
10	51.80	42.17	1.36	1.74	2.95	101.80
11	95.80	1.77	0.99	0.18	0.11	97.90
12	96.40	0.86	2.20	0.32	0.31	97.40

In these investigations porosity en masse and specific gravity were determined by the method outlined by Hillebrand (6, p. 51-53). Hygroscopic capacity was taken as a measure of the porosity of the finely ground material. It was determined by drying the sample to constant weight in an oven at 110°C. and determining the amount of water absorbed when placed over 10-per-cent H<sub>2</sub>SO<sub>4</sub> for two weeks.

Hardness was determined by measuring the abrasion which took place when particles between 3- and 4-mesh in size were rotated in a pebble mill; 100 gm. of material were used, with rotation in the mill for 2 hours. At the end of this period the fraction which passed a 10-mesh screen was determined.

TABLE 4  
*Solubility of limestone materials in 2 N acetic acid*

NUMBER	AMOUNT REACTING IN 30 MINUTES	
	50 mesh	100 mesh
	<i>per cent</i>	<i>per cent</i>
1	71.4	79.6
2	64.6	76.0
3	10.4	11.2
4	63.8	77.6
5	57.6	66.6
6	50.8	52.4
7	51.0	62.4
8	60.8	75.0
9	6.4	7.6
10	6.2	8.2
11	50.0	61.0
12	48.0	50.6

TABLE 5  
*Effect of application of limestone materials upon  $\text{CaCO}_3$  requirement of acid soils, per 2,000,000 pounds*

NUMBER	TRUMBULL SILT LOAM			DEKALE SANDY LOAM	
	4th day	11th day	35th day	6th day	20th day
	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>
50-mesh separate					
1	1812	1561	1500	2937	656
2	1750	1125	937	2531	625
3	2437	2250	2125	4656	2906
4	1787	1287	1000	2906	812
5	2031	1812	1625	3469	1219
6	2375	1825	1656	4219	2062
7	2312	2000	1750	4190	2000
8	2000	1625	1500	3000	1125
9	2406	2375	2310	5125	3562
10	2406	2394	2250	5000	3219
11	1781	1337	1062	2781	875
12	2194	1938	1781	3594	1375
100-mesh separate					
1	1094	781	375	750	281
2	844	562	190	656	220
3	2187	1812	1060	2812	1375
4	969	656	250	844	406
5	1375	937	625	1594	437
6	1812	1312	750	2375	656
7	1687	1125	689	1937	625
8	1594	912	437	1469	375
9	2219	1842	1125	3500	1669
10	1919	1594	1060	3031	1562
11	1500	719	406	1125	437
12	1562	1062	562	1531	500

In the solubility investigations two sizes of material were used; first, that which passed a 40-mesh and was retained by a 50-mesh screen; second, that which passed an 80-mesh and was retained by a 100-mesh screen. These separates were thoroughly washed on the screen to insure freedom from finer material.

It was believed that the relative solubilities of the various materials could be satisfactorily determined by measuring their rate of reaction in an excess of dilute acetic acid of standard strength during a definite time interval. Acetic acid was chosen on account of its low dis-

TABLE 6

*Residual carbonates calculated as  $\text{CaCO}_3$  after application of limestone materials to acid soils*

NUMBER	$\text{CaCO}_3$ PER 2,000,000 LBS. SOIL			
	Trumbull silt loam—35th day		DeKalb sandy loam—20th day	
	Residual	Neutralized	Residual	Neutralized
	lbs.	lbs.	lbs.	lbs.
50-mesh separate				
1	6430	3570	9040	10960
2	5570	4430	8860	11140
3	7950	2050	13880	6120
4	6540	3460	9590	10410
5	7270	2730	10340	9660
6	7640	2360	12070	7930
7	7390	2610	12160	7840
8	6640	3360	10180	9820
9	8290	1710	14430	5570
10	8130	1870	14270	5730
11	6090	3910	9950	10050
12	6920	3180	10470	9630
100-mesh separate				
1	3140	6860	7620	12380
2	2930	7070	6460	13540
3	5040	5960	10320	9680
4	3000	7000	7230	12770
5	4090	5910	7960	12040
6	4630	5370	8320	11680
7	4160	5840	8390	11610
8	3250	6750	7680	12320
9	5220	4780	11160	8840
10	5090	4910	10980	9020
11	3020	6980	7350	12650
12	4090	5910	8000	12000

sociation ( $K = 1.8 \times 10^{-9}$ ), which permits considerable change in the total acid present without altering appreciably the hydrogen-ion concentration of the solution; it was desirable in this work to keep this factor as nearly constant as possible.

Method employed: A sample of limestone containing the equivalent of 5 gm. of  $\text{CaCO}_3$  was placed in a 300-cc. Erlenmeyer flask, 50 cc. of 2 N acetic acid were added and the flask was kept in constant agitation for 30 minutes to avoid irregularities of diffusion. At the end of this period the liquid in the flask was filtered as quickly as possible, and a 5-cc. aliquot titrated with 0.2 N NaOH, using bromthymol-blue as the indicator. From this titration



the amount of limestone reacting with the acid during the period was calculated. The change in concentration of the acid was less than pH 1, as determined colorimetrically.

To determine the solubility of the limestone particles when added to an acid soil, two soils were employed, a Trumbull silt loam and a DeKalb silt loam, having limestone requirements of 3,200 and 6,100 lbs. respectively. A modification of the Jones lime requirement method (7) was used, wherein bromthymol-blue was substituted for phenolphthalein as the indicator, and the  $\text{CaCO}_3$  requirement was calculated directly from the titration, rather than by using the arbitrary factor of 1.8. To insure a sufficient excess of limestone to neutralize all the acidity present, applications were made to the soils at rates equivalent to 10,000 and 20,000 lbs. of  $\text{CaCO}_3$  per 2,000,000 lbs. of soil. The limestone particles were thoroughly mixed with the dry soil and sufficient water was added to obtain the optimum moisture-content. It was found that the progress of the reaction could be observed by determining the  $\text{CaCO}_3$  requirement by the foregoing method. A rubber pestle was used in mixing of the soil with the calcium acetate to prevent mechanical disintegration of the limestone particles. As a check upon the results thus obtained, the Truog (10) test was used. A very close correlation was found between the results of the Jones and Truog methods.

#### RATE OF SOLUBILITY IN ACID SOILS, AS MEASURED BY RESIDUAL CARBONATES

In the decomposition of limestone in an acid soil, the carbonates are, presumably, neutralized as rapidly as they go into solution. As pointed out by McIntire (8) the calcium and magnesium, more especially the latter, may react with the more readily soluble soil silicates with the formation of the corresponding alkaline earth silicates. In either case, a determination of the carbonates remaining in the soil at the end of a given time interval after treatment should afford a measure of the rate of solubility of the limestone materials under investigation.

With this idea in mind, residual carbonates were determined in the soils treated as previously described. A modification of Bear and Salter's (3, p. 21-22) method was used, whereby the carbonates were decomposed by treatment with strong acid, and the  $\text{CO}_2$  liberated was absorbed by soda-lime contained in a Fleming absorption bulb. With the large quantity of soil used (20 gms.) this method proved to be more satisfactory than the volumetric procedure involving the absorption of the gas in a  $\text{NaOH}$  solution. Results are given in table 6.

#### DISCUSSION OF RESULTS

The limestone materials studied varied considerably in chemical composition and physical properties. It should be noted that nine of the twelve samples were from quarries producing agricultural ground limestone, so that any significant differences in their rates of solubility should be of special importance.

In a study of the data, the slower rate of reaction of the dolomitic materials is the most noticeable feature. This is clearly shown in table 4 where it is seen that the three typical dolomites no. 3, 9 and 10, are very slowly soluble in 2 *N* acetic acid, having an initial hydrogen-ion concentration of pH. 3.2

comparable to a very acid soil, while the high-calcium limestones, even though very hard and compact, were readily soluble. In the same table it is of interest to note that with both sizes of separates no. 11, a soft travertine, was much more slowly dissolved by the acid, then were nos. 1, 2 and 4, all of which were hard and compact high-calcium limestones.

Figures 1 and 2 shows in graphic form the results of the treatment of the DeKalb soil with 50 and 100-mesh material. A study of these graphs, in connection with results shown in table 5 disclose the following relationships:

1. There is a fairly close agreement in the order of solubility of the limestones for the different sized separates, at different intervals of time after application, and with the two different soils used. This order of solubility is in close agreement with that found in acetic acid, as shown in table 4.

2. In general, the rate of solubility seems to be dependent upon the relative percentages of magnesium and calcium carbonates in the stone. The six stones containing no appreciable amount of  $MgCO_3$  are the more effective in decreasing the  $CaCO_3$  requirement, while the three dolomites are in every case much slower in their action.

3. With corresponding applications of limestone material, the rate of reaction is much more rapid in the case of the more acid DeKalb sandy loam. This is probably due to differences in the initial hydrogen-ion concentration of the soil solutions. Prior to treatment, the Trumbull silt loam showed a reaction of pH 5.6 while that of the DeKalb sandy loam was pH 4.2.

4. The 100-mesh material brought about a more rapid rate of decrease in acidity in both soils used. The factor of fineness of grinding is seen to be of decided importance in overcoming the differences in solubility due to the nature of the stone. As applied to the Trumbull silt loam, the solubility of the 50-mesh dolomitic material is very slow and becomes much less rapid with increasing time. The 100-mesh high-calcium material, added to the same soil, reacts much more slowly as the neutral point is approached, while the high-magnesium stones of the same degree of fineness continue to reduce the  $CaCO_3$  requirement at an almost uniform rate. When the materials are applied to the very acid DeKalb sandy loam, a more rapid chemical disintegration of the particles takes place, and the differences in solubility of the materials used are of a smaller order of magnitude. The 100-mesh particles of all the stones tended to bring the reaction rapidly toward the neutral point. Only for the three dolomites is there any significant  $CaCO_3$  requirement remaining at the end of the 20-day period.

5. With the exception of the coarser separate of dolomitic material when applied to the less acid soil, the rate of reaction in all cases is fairly rapid. Thus in 20 days the 100-mesh material reduced the  $CaCO_3$  requirement of the DeKalb sandy loam to one-twelfth the original amount.

Figure 3 shows graphically a comparison between the differences in solubility of the limestone materials used and their physical and chemical properties. The adverse effect of relatively large amounts of  $MgCO_3$  upon the rate of solubility is clearly shown. Further than this it would seem impossible to draw any conclusions. The following examples of irregularity are significant: no. 2, a high-calcium stone, very hard and only slightly porous in the mass, is the most rapidly soluble in practically all the tests. Particles used seem to show a relatively high degree of hygroscopicity, indicating a porosity in the ultimate grain rather than in the mass. On the other hand, no. 8, also a

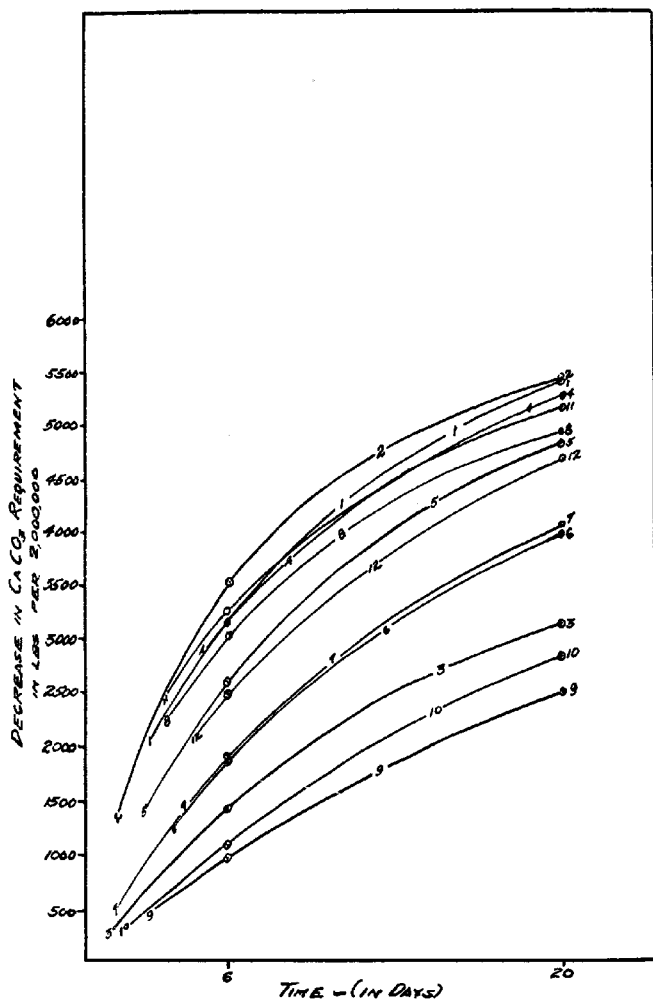


FIG. 1. EFFECT OF APPLICATION OF 50-MESH LIMESTONE MATERIALS UPON  $\text{CaCO}_3$  REQUIREMENT OF ACID DEKALB SANDY LOAM

Numbers refer to limestone samples. Curves indicate effect of material in reducing the acidity at the end of a given time interval after application. Readings taken as indicated, on 6th and 20th days.

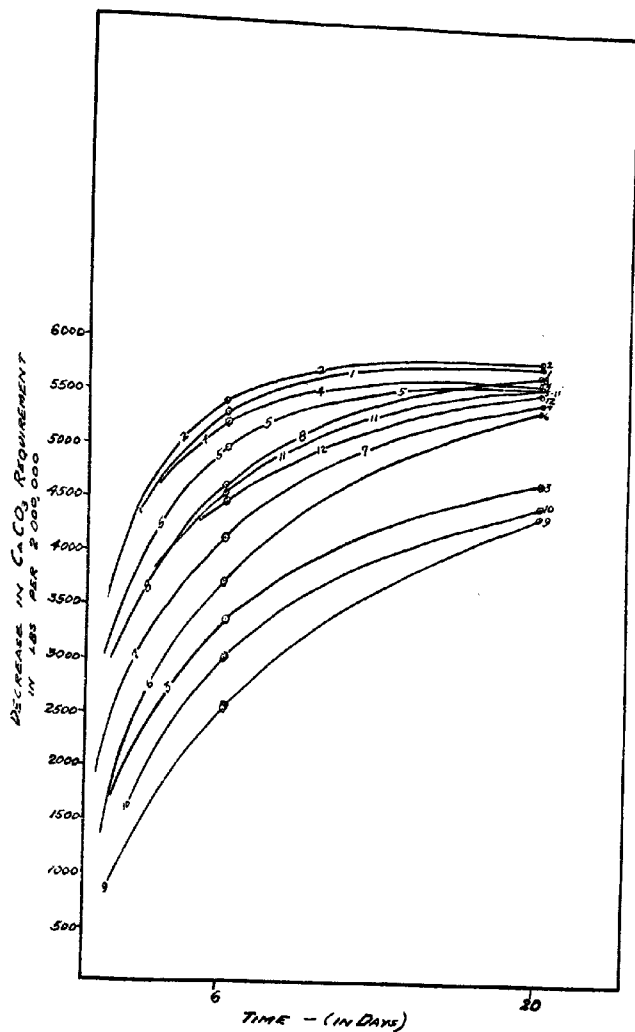


FIG. 2. EFFECT OF APPLICATION OF 100-MESH LIMESTONE MATERIALS UPON  $\text{CaCO}_3$  REQUIREMENT OF ACID DEKALB SANDY LOAM

Numbers refer to limestone samples. Curves indicate effect of material in reducing the acidity at the end of a given time interval after application. Readings taken as indicated, on 6th and 20th days.

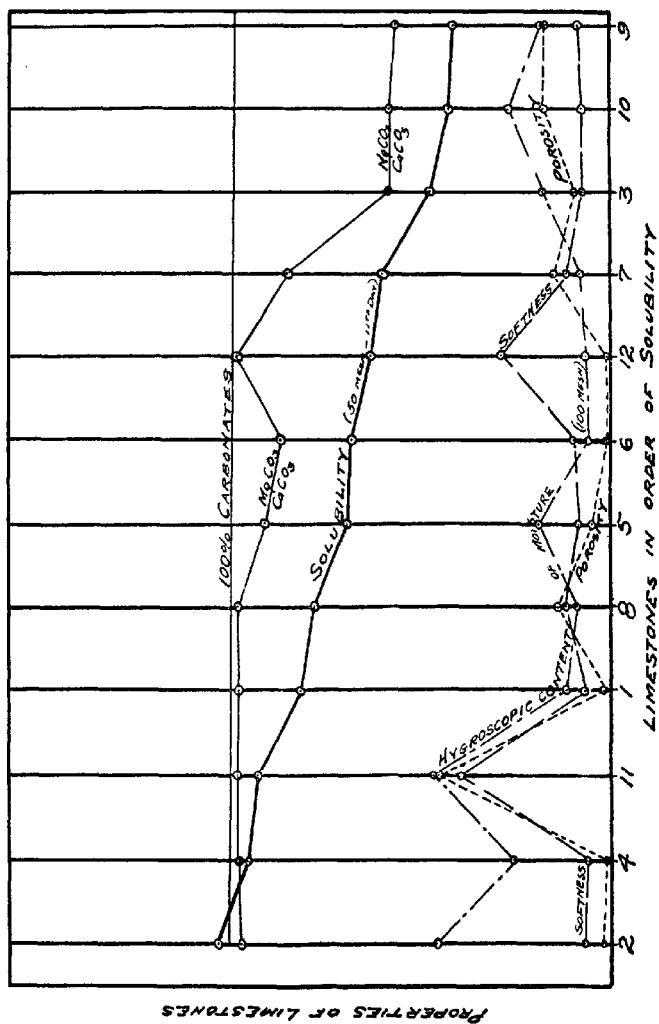


FIG. 3. COMPARISON OF SOLUBILITIES OF LIMESTONE MATERIALS STUDIED AND THEIR CHEMICAL COMPOSITION AND SOME PHYSICAL PROPERTIES. Numbers refer to limestone samples. The relative proportion of  $MgCO_3$  and  $CaCO_3$  on the basis of 100 per cent carbonates is shown by two upper lines. Other lines are on different abscissa, depending upon unit of measurement.

high-calcium stone, is much more slowly soluble, though softer, more porous in the mass, but with a smaller hygroscopic capacity. No. 12, a crystalline marble containing fairly coarse grains of practically pure calcite, is less readily soluble than any of the high-calcium limestones, although in the rock mass it is quite easily crushed and abraded.

It is of interest, though not necessarily significant, to note that the five stones which break into crystalline fragments are among the seven stones which are the least soluble. It happens that all but one of these are high in magnesium, two being typical dolomites.

In determining the residual carbonates after the  $\text{CaCO}_3$  requirement had been materially reduced a rather definite relationship was found between the  $\text{CaCO}_3$  requirement obtained by direct titration in the Jones method and the decrease in carbonates. It is thus seen that there is a very constant relation-

TABLE 7

*Comparison of decrease in Jones  $\text{CaCO}_3$  requirement with decomposition of carbonates*

NUMBER	TRUMBULL SILT LOAM (100-MESH MATERIAL) ON THE 35TH DAY		
	$\text{CaCO}_3$ equivalent decomposed	Decrease in Jones $\text{CaCO}_3$ requirement	Factor
1	6860	2875	2.38
2	7070	3000	2.35
3	4955	2140	2.31
4	7000	2950	2.36
5	5910	2575	2.29
6	5365	2450	2.31
7	5840	2511	2.32
8	6750	2763	2.44
9	4780	2075	2.30
10	4910	2140	2.29
11	6980	2794	2.41
12	5910	2638	2.24

ship between the results given by the Jones method and the absolute lime requirement, as shown by the soil's ability to liberate carbon dioxide from calcium and magnesium carbonates. Complete data show a similar relationship for all the results obtained with both separates for each soil but for the sake of brevity were not included.

It is significant that the results of other measurements have been so well verified by the determination of residual carbonates. So far as has been observed, there has been no tendency for the dolomitic limestones to be decomposed through conversion into magnesium silicate at a more rapid rate than the high-calcium limestones. These observations are apparently at variance with results reported by McIntire (8), but it should be noted that he worked with material passing a 100-mesh screen, which may have contained a large amount of very fine particles. The relatively greater solubility of the 100-

mesh separate from the dolomitic stones might indicate a tendency similar to that observed by McIntire. It must also be remembered that in the present work the neutral point was not reached and while any acidity exists, no appreciable formation of magnesium silicate would be expected.

The results relative to the slower rate of reaction of the dolomitic stones are in agreement with the findings of Ames and Schollenberger (1) and White and Gardner (11, p. 16).

#### CONCLUSIONS

With particles of the size ordinarily found in agricultural ground limestone, there is no apparent relationship between the rate of solubility in acid soils and any physical property of the rock material.

The rate of solubility is very largely influenced by the relative amount of  $\text{MgCO}_3$  in the material. With coarse particles, the decreased solubility may be of considerable significance. The finer the material is ground, the less important this factor becomes, and with limestone containing considerable 100-mesh material, it is believed that the rate of solubility of dolomitic stones would be sufficiently rapid for all practical purposes.

#### SUMMARY

1. A study has been made of twelve limestone materials, covering a wide variation in physical and chemical properties, with the purpose of determining the factors influencing their rates of solubility when applied to acid soils.

2. The materials used were tested for hardness, porosity en masse, porosity of the 50- and 100-mesh separates as determined by hygroscopic capacity, specific gravity, crystalline composition and chemical analysis.

3. The rate of solubility of 50- and 100-mesh separates of the materials used was determined: first, by solubility in 2*N* acetic acid during 30 minutes; second, by measuring the limestone requirement and residual carbonates at definite intervals after their application to two soils of different degrees of acidity.

4. There was no apparent relationship between any physical properties determined and the rate of solubility.

5. The limestones used varied greatly in their rate of solubility, but showed practically the same order of solubility by all of the methods employed.

6. The differences in solubility of limestones containing appreciable amounts of  $\text{MgCO}_3$  could be explained by the lower solubility of dolomite in a solution having a hydrogen-ion concentration comparable to that prevailing in an acid soil.

7. The differences in solubility of limestones containing no appreciable amount of  $\text{MgCO}_3$  could not be explained with the data obtained in these investigations.

8. Further study upon differences in solubility of high-calcium limestones of similar chemical composition seems desirable.

9. The fineness to which dolomitic limestones are ground is of greater relative importance than is the case with high-calcium stones.

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## A COMPARISON OF MAGNESIAN AND NON-MAGNESIAN LIMESTONE IN SOME 5-YEAR ROTATIONS<sup>1</sup>

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In 1908 an experiment was laid out for the purpose of testing two sources of lime, applied in different amounts and in connection with different crop rotations (3, 4). The materials used were magnesian (dolomitic) and non-magnesian limestones, each applied at the rate of 1000, 2000 and 4000 pounds per acre. For carrying out the work twenty-eight  $\frac{1}{8}$ -acre plots were divided into four blocks of seven plots each on which four different five-year rotations have been carried out. With the season of 1922, three 5-year periods have been completed. The rotations are as follows:

No. 1. Corn, oats, wheat, and two years of timothy and clover.

No. 2. Corn, potatoes, rye, and two years of timothy and clover.

No. 3. Corn, potatoes, tomatoes, lima beans, cucumbers. This has been called the market garden rotation.

No. 4. Corn; oats and peas, millet; rye and vetch, rape; rye or rye and vetch, cowpeas or soybeans; oats and peas, cowpeas or soybeans. This has been designated as a forage crop rotation.

The plan provides for seeding a legume cover crop between the main crops wherever possible, and it will be noted that at least one legume was introduced in each rotation. The forage crop rotation provides for at least one legume every year.

Acid phosphate and muriate of potash have been applied annually, the former at the rate of 300 to 400 pounds per acre and the latter at 100 to 200 pounds. In most cases commercial nitrogenous fertilizers were applied in amounts to furnish nitrogen equivalent to 160 to 200 pounds of nitrate of soda per acre. In some instances such as the market garden crops, heavier applications have been made. Less nitrogen has generally been used for the forage crop rotation than for the others. No farm manure has been used during the entire 15 years.

The lime requirement of this soil was not determined when the experiment was started, but determinations made on samples from the check plots at the end of the first five years, showed a requirement of approximately 1000 to 1500 pounds of lime (CaO) per acre, whereas the plots that had in 1908 received

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2000 and 4000 pounds per acre were, at the end of the first 5 years, close to the neutral point.

Careful crop records have been kept and in practically all cases samples of the dry material have been analyzed for nitrogen, so that a record might be had of the amount of nitrogen taken off the land in the form of crops.

The results of this work are conveniently shown by reporting the yields for the crops by years, with averages for the three years of each crop. In the case of corn and grain crops, the yields are reported as total dry matter in the grain and stover or straw as the case may be; the hay crops are reported as dry matter, and potatoes, tomatoes and cucumbers as marketable crops.

#### ROTATION 1 (PLOTS 21-27)

The results for rotation 1 are shown in table 1. It may be explained here that following the oat crop of 1909 cowpeas were sown as a cover crop but these made rather poor growth apparently for lack of thorough inoculation. Also following the wheat crop of 1910, cowpeas were seeded as a green manure crop and made fair growth before being plowed under in preparation for seeding timothy and clover. The timothy and clover was seeded in the fall of 1910 but the crop was a failure and this necessitated the seeding of oats in the spring of 1911. This explains the figures for oats in 1911 where the yields of timothy and clover should have appeared.

With very few exceptions the lime treatments resulted in some increase for each crop. In some cases this increase amounted to only a few pounds while in others it amounted to as much as 500 to 1000 pounds of dry matter per acre for the one-half ton application of limestone. In most cases the 1-ton application gave a fair increase over the half-ton application, but the 2-ton application gave either no increase or only a small increase over the 1-ton application.

Of the five crops the oats showed the least response to the lime treatment and the timothy and clover the greatest. In 1921 the plot which receives one ton of magnesian limestone yielded more than twice as much hay as the no-lime plot, and in 1922 the same plot yielded more than three times as much. In 1916 and 1922 a second cutting of hay was obtained from these plots while in 1917 and 1921 only one cutting was obtained.

Attention may be drawn to the fact that in every case one ton of magnesian limestone gave larger yields than two tons of the calcium limestone, and with slight exception as large or larger yields than the two tons of magnesian limestone.

In 11 out of 15 years one-half ton of magnesian limestone gave larger yields than one ton of calcium limestone, the general average for the former being 3721 pounds and for the latter 3449 pounds. A little figuring will show that in most cases the increases obtained with one-half ton of both forms of limestone were sufficient to make its use profitable.

Taking the five crops for three years each—15 years in all—the  $\frac{1}{2}$ -ton application of magnesian limestone gave an average increase of approximately 1200

pounds of dry matter per acre; the 1-ton application of magnesian limestone an average increase of approximately 1400 pounds per acre and the 2-ton

TABLE 1  
*Yield of dry matter in crops in rotation 1 (acre basis)*

YEAR	NO LIME	½ TON		1 TON		2 TONS	
		Ca limestone	Mg limestone	Ca limestone	Mg limestone	Ca limestone	Mg limestone
Corn (grain and stover)							
	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
1908	4673	4582	5842	4935	5241	5205	4846
1913	3500	4025	5350	4350	5375	4825	5200
1918	4593	4876	5271	5189	5434	5208	5721
Average.....	4255	4494	5488	4825	5350	5079	5256
Oats (grain and straw)							
1909	1524	1572	2056	1560	2212	1917	2345
1914	1520	1280	1660	1640	1720	1620	1680
1919	2320	3120	3120	3080	3400	3280	3360
Average.....	1788	1991	2279	2093	2444	2272	2462
Wheat (grain and straw)							
1910	2850	3175	4200	3800	4325	4100	4375
1915	2280	2640	2840	2900	2940	2720	2820
1920	1040	2360	2620	2440	2800	2580	2620
Average.....	2057	2725	3220	3047	3355	3133	3272
Timothy and clover (oats 1911)							
1911	2100	2100	2800	2400	2650	2500	3000
1916	3800	4660	5540	5800	6540	6060	6340
1921	1700	2860	3420	2760	3680	2840	3560
Average.....	2533	3207	3920	3653	4290	3800	4300
Timothy and clover							
1912	1925	2200	2875	2250	3200	2175	3275
1917	2620	3520	3520	3640	3800	3500	4220
1922	1736	3824	4700	4984	5610	5310	6040
Average.....	2094	3181	3698	3625	4203	3662	4512
GENERAL AVERAGE...	2545	3120	3721	3449	3928	3589	3960

application an average increase of a little over 1400 pounds per acre. The average increases for the calcium limestone treatments were slightly below those for the magnesian limestone.

The results obtained in this rotation give little basis for the use of as much as two tons of limestone, and indeed there may be some question as to the advisability of using more than one-half ton, especially where magnesian limestone is being applied.

*The yield of nitrogen*

Table 2 shows the yields of nitrogen for this rotation based on the dry weight of the crop and the nitrogen content of a representative sample. For a given crop, the yield of dry matter is, in most cases an index of the yield of nitrogen.

TABLE 2  
*Yield of nitrogen per acre in different crops in rotation*

CROP	YEAR	NO LIME	½ TON		1 TON		2 TONS	
			Ca lime- stone	Mg lime- stone	Ca lime- stone	Mg lime- stone	Ca lime- stone	Mg lime- stone
First 5-year period, 1908-1912								
Corn	1908	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
Oats	1909	38.5	42.2	56.1	47.2	51.5	49.8	52.4
Wheat	1910	17.6	18.3	24.8	17.9	27.4	22.4	29.6
Oats	1911	27.5	27.7	38.5	35.9	38.7	36.6	38.9
Timothy and clover	1912	30.1	28.5	40.0	32.3	38.9	34.0	43.0
Average		16.3	18.8	25.7	18.7	28.4	19.3	29.8
		26.0	27.1	37.0	30.4	37.0	32.4	38.7
Second 5-year period, 1913-1917								
Corn	1913	25.6	33.1	41.2	40.3	52.3	49.3	54.2
Oats	1914	19.7	17.0	21.5	22.2	23.4	20.8	24.0
Wheat	1915	24.1	28.3	30.5	31.5	29.6	28.7	29.7
Timothy and clover	1916	35.5	66.3	74.7	88.6	106.1	97.6	99.8
Timothy and clover	1917	19.9	28.5	27.8	30.9	32.3	29.7	34.6
Average		25.0	34.7	39.1	42.7	48.7	45.2	48.5
Third 5-year period 1918-1922								
Corn	1918	48.3	54.8	60.3	58.5	62.6	57.7	54.8
Oats	1919	24.4	34.4	35.7	35.8	42.1	36.0	41.5
Barley	1920	12.9	25.3	28.6	26.1	31.2	28.9	31.2
Timothy and clover	1921	14.9	22.3	26.2	23.9	30.6	24.5	28.4
Timothy and clover	1922	16.6	32.7	56.3	60.4	60.7	76.5	86.6
Average		23.4	33.9	41.4	41.0	45.4	44.7	48.5

With only a few exceptions the corn returned approximately 40 to 50 pounds of nitrogen per acre. The returns for the wheat and oats were considerably under these figures. The yields from the hay crop were very much influenced by the clover. In 1916 there was a second cutting most of which was clover

and the total yield of nitrogen was high. In 1917 there was only one cutting with a thin stand of clover and this was reflected in a low nitrogen yield. This was true also of the 1921 hay crop. In 1922 the second cutting of the hay accounted for a nitrogen yield of more than 60 pounds per acre for the 1- and 2-ton applications of limestone. In 1922 the plot that received two tons of magnesian limestone yielded more than five times as much nitrogen as the check plot. In 1916 the 1-ton application of magnesian limestone yielded three times as much nitrogen as the check. With but one exception the plots receiving 1-ton of magnesian limestone yielded more nitrogen than those receiving 2-ton of calcium limestone.

It is of interest to note the close agreement between the average yields for the second and third 5-year periods which range from about 25 pounds for the check plot to nearly 50 pounds for the 1- and 2-ton applications of magnesian limestone. In this connection it may be explained that there was very little clover on the check plot. The low average yield of nitrogen for the first 5-year period is due largely to the fact that in 1911 the timothy and clover were replaced by oats.

#### ROTATION 2 (PLOTS 28-34)

As in the case of rotation 1, the timothy and clover which was seeded in 1910 failed and was replaced by oats in the spring of 1911. The crop yields are shown in table 3. The lime treatments again showed some increase over the check plot in the majority of cases. The potatoes for 1909 (omitted from average), the rye for 1915 and the oats for 1911 were exceptions. The failure of the lime plots to show increases in 1911 was undoubtedly due to the failure of the timothy and clover and the substitution of oats, mention of which has already been made. The potato yields were somewhat irregular but in the majority of cases the lime-treated plots showed some increase over the check plot. The yields for this crop were all low; in 1909 it was less than one-fifth of the average for the other two years. The records fail to explain the reason of this failure.

An examination of the average yields for the five crops—15 years in all—shows that  $\frac{1}{2}$ -ton of magnesian limestone caused an average increase of about 625 pounds of dry matter per acre over the check plot. One ton of magnesian limestone caused an average increase of only 124 pounds more than the  $\frac{1}{2}$ -ton application; the 2-ton application caused an average increase of only 75 pounds more than the  $\frac{1}{2}$ -ton application. In 10 years out of the 15, one ton of calcium limestone gave larger yields than an equal amount of magnesian limestone. In this rotation as in the previous one, the timothy and clover showed the greatest response to the lime. Taking the general averages the calcium limestone gave slightly higher yields than the magnesian limestone.

TABLE 3  
Yield of crops in rotation 2 (acre basis)

YEAR	NO LIME	$\frac{1}{2}$ TON		1 TON		2 TONS	
		Ca limestone	Mg limestone	Ca limestone	Mg limestone	Ca limestone	Mg limestone
Corn (grain and stover)							
	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
1908	4800	6005	4745	5500	4423	5311	4961
1913	3475	4425	4700	4325	4750	4750	4725
1918	4883	5058	5358	5799	5294	5845	5696
Average.....	4386	5163	4934	5208	4822	5302	5127
Potatoes							
1909*	1000	1020	1020	880	840	920	840
1914	6200	7040	5960	7100	5620	6560	5240
1919	5406	5615	6380	6695	6635	5760	6020
Average.....	5803	6328	6170	6898	6128	6160	5630
Rye (grain and straw)							
1910	5425	6650	7150	5850	7500	6700	8050
1915	4080	3960	4040	4100	4320	3840	3880
1920	3920	4800	4760	5080	5000	5680	6040
Average.....	4475	5137	5317	5010	5607	5407	5990
Timothy and clover (oats 1911)							
1911	2725	2850	2900	2650	2900	2700	2600
1916	4580	5280	5700	5980	6200	6100	5860
1921	2864	3348	2880	3570	3470	2840	3500
Average.....	3390	3826	3827	4067	4190	3880	3987
Timothy and clover							
1912	2900	3650	3650	3425	3225	3800	3150
1917	3500	3680	3960	4440	3840	4500	3580
1922	3600	5360	5180	6800	6082	5680	5720
Average.....	3333	4230	4263	4888	4382	4660	4150
GENERAL AVERAGE...	4277	4937	4902	5214	5026	5082	4977

\* Omitted from average.

*The yield of nitrogen*

Table 4 shows the total yield of nitrogen per acre for the crops of this rotation and the averages for the three 5-year periods. These figures follow the crop yields with a fair degree of regularity although there are some exceptions. Without exception the 5-year averages show a larger yield of nitrogen for the

TABLE 4  
*Yield of nitrogen per acre in different crops in rotation 2*

CROP	YEAR	NO LIME	½ TON		1 TON		2 TONS	
			Ca lime- stone	Mg lime- stone	Ca lime- stone	Mg lime- stone	Ca lime- stone	Mg lime- stone
First 5-year period, 1908-1912								
Corn .....	1908	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
Potatoes* .....	1909	44.0	63.3	50.3		42.6	55.5	47.7
Rye .....	1910	3.7	3.6	3.6	3.3	3.2	3.4	3.4
Oats .....	1911	32.4	40.6	44.4	38.2	49.1	47.6	55.1
Timothy and clover .....	1912	35.7	41.2	39.7	38.2	41.8	36.0	37.8
		24.8	33.5	31.5	30.4	30.7	36.1	30.2
Average .....		34.2	44.7	41.5	35.6	41.1	43.8	42.7
Second 5-year period, 1913-1917								
Corn .....	1913	33.3	50.6	56.5	61.2	62.1	70.4	60.9
Potatoes .....	1914	22.5	23.8	21.7	20.9	21.1	20.3	19.3
Rye .....	1915	28.2	26.7	29.4	29.5	30.6	28.8	28.5
Timothy and clover .....	1916	53.9	61.7	79.2	82.7	90.8	101.9	96.6
Timothy and clover .....	1917	27.3	29.4	34.4	39.0	37.5	36.0	34.7
Average .....		33.0	38.5	44.2	46.7	48.4	51.5	48.0
Third 5-year period, 1918-1922								
Corn .....	1918	55.0	52.3	67.0	63.7	60.0	67.3	69.3
Potatoes .....	1919	16.9	23.8	27.9	26.8	20.3	23.3	25.2
Rye .....	1920	27.4	29.3	32.9	31.5	34.5	37.2	43.0
Timothy and clover .....	1921	24.8	31.2	31.4	44.5	47.8	35.2	51.1
Timothy and clover .....	1922	34.7	64.5	62.7	87.6	96.8	80.1	72.8
Average .....		31.8	40.2	44.4	50.8	51.9	48.6	52.3

\* Omitted from average.

lime-treated plots than for the check plot. In the majority of cases the magnesian limestone gave slightly higher yields of nitrogen than the calcium limestone. It will be recalled from table 3 that the general averages for crop yields were slightly higher for the calcium limestone than for the magnesian limestone. This apparent discrepancy is undoubtedly due to the fact that in



a number of cases the percentage of nitrogen was higher in the dry matter from plots treated with magnesian limestone.

As in the case of rotation 1 the potato crop for 1909 was abnormally small and for this reason the nitrogen figures have here also been omitted from the average. In 1916 the timothy and clover yielded a second cutting which was reflected in the large amount of nitrogen recovered for that year. Only one cutting was taken in 1917 and this did not contain very much clover. The crop was small again in 1921 but in 1922 two cuttings were obtained. The yield of nitrogen for this year was 96.8 pounds per acre with one ton of magnesian limestone. The highest yield in 1916 was 101.9 pounds with two tons of calcium limestone. This is almost twice as much as was returned from the check plot this year. In the matter of nitrogen recovery for the 15-year period, one ton of limestone proved almost as effective as two tons.

#### ROTATION 3 (PLOTS 35-41)

The corn used here has been field corn and not sweet corn. It has been the practice to seed a green manure crop after the main crop. In most cases this green manure crop has been rye and vetch, or rye, vetch and clovers. These crops have aided materially in maintaining the supply of nitrogen and organic matter. No farm manure has been used. Somewhat more commercial nitrogen has been used for vegetable crops than for the general farm crops of rotations 1 and 2.

The yields for the crops of this rotation for the 15 years are shown in table 5. With only a few exceptions the lime-treated plots gave larger returns than the check plots. The lima beans and cucumbers showed the greatest response to the lime treatment. With two or three exceptions the potatoes also showed a good response but the yields were generally low. The 1909 crop, like that of rotation 2, was exceptionally small and the figures have been omitted from the averages.

The results for the tomatoes are somewhat irregular but distinct gains with the lime treatment are shown for 1910 and 1915. In 1920 the results are more irregular, the no-lime plot giving the highest yields.

Taking the 15-year averages, the 1- and 2-ton applications of magnesian limestone show somewhat higher yields than the same amounts of calcium limestone, but with the  $\frac{1}{2}$ -ton application the order is reversed.

It is quite evident that the use of lime in this rotation has proved profitable, but it is also evident that the 2-ton application is excessive. Indeed, the general averages show that the  $\frac{1}{2}$ -ton application has given almost as good yields as the 1-ton application and better than the 2-ton application. The results indicate that the 2-ton magnesian limestone treatment had a depressing effect on the lima beans in each of the three years and on the cucumbers in 1912 and 1922.

TABLE 5  
*Yield of crops in rotation 3 (acre basis)*

YEAR	NO LIME	½ TON		1 TON		2 TONS	
		Ca limestone	Mg limestone	Ca limestone	Mg limestone	Ca limestone	Mg limestone

<i>Corn (grain and stover)</i>							
	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
1908	4792	4706	4537	4401	4953	5333	5206
1913	4125	4525	4750	4875	5375	5350	5400
1918	5242	5788	5435	6058	5820	5957	5915
Average.....	4720	5006	4907	5111	5383	5547	5507

<i>Potatoes</i>							
1909*	640	600	740	720	880	760	690
1914	4800	6320	5680	6300	6260	5740	5100
1919	7165	10310	5425	9590	11445	7470	10355
Average.....	5982	8315	5552	7945	8852	6605	7727

<i>Tomatoes †</i>							
1910	13455	21218	19043	19210	21688	17670	18973
1915	24080	28550	25454	27860	27720	25610	28924
1920	18728	15034	16664	13768	17747	9899	16352
Average.....	18754	21601	20387	20279	22385	17726	21416

<i>Lima beans (dry shelled)</i>							
1911	540	800	740	750	750	715	620
1916	480	660	540	860	660	720	360
1921	380	620	1050	950	1330	1370	950
Average.....	467	693	777	853	913	935	643

<i>Cucumbers</i>							
1912	5300	8000	7500	9300	5300	8800	4500
1917	8138	11618	13432	13536	13482	11688	13496
1922	5218	8948	9228	9496	9110	12726	8408
Average.....	6219	9522	10053	10777	9297	11071	8801
GENERAL AVERAGE.....	7228	9027	8335	8993	9366	8377	8819

\* Omitted from average.

† These yields have been previously reported by Blair (1). In that paper the calculations for 1910 were, unfortunately, made on incomplete data. The figures given here are based on complete data.

*The yield of nitrogen*

The nitrogen returns in the different crops for the three 5-year periods for this rotation are shown in table 6. There is a fairly close agreement between

TABLE 6  
*Yield of nitrogen per acre in different crops in rotation 3*

CROP	YEAR	NO LIME	½ TON		1 TON		2 TONS	
			Ca lime- stone	Mg lime- stone	Ca lime- stone	Mg lime- stone	Ca lime- stone	Mg lime- stone
<i>First 5-year period, 1908-1912</i>								
Corn .....	1908		lbs. 41.5	lbs. 45.5	lbs. 42.7	lbs. 44.9	lbs. 49.8	lbs. 50.7
Potaoties*	1909		2.4	2.3	2.9	2.9	3.4	3.2
Tomatoes†	1910		24.2	38.2	34.3	34.6	39.0	31.8
Lima beans.....	1911		19.5	28.0	26.3	28.1	27.7	25.8
Cucumbers‡	1912		5.8	8.8	8.3	10.2	5.8	9.7
<i>Average</i> .....			22.8	30.1	27.9	29.5	30.6	29.3
<i>Second 5-year period, 1913-1917</i>								
Corn .....	1913		43.0	49.1	46.5	53.4	56.3	62.1
Potatoes .....	1914		15.4	16.2	16.4	19.1	19.3	20.7
Tomatoes.....	1915		40.6	48.1	42.9	46.9	46.7	43.1
Lima beans.....	1916		16.7	22.4	19.3	29.4	23.2	22.8
Cucumbers .....	1917		9.5	14.8	16.9	18.3	16.9	15.0
<i>Average</i> .....			25.0	30.1	28.4	33.4	32.5	31.4
<i>Third 5-year period, 1918-1922</i>								
Corn .....	1918		58.5	68.7	69.9	72.0	70.0	72.9
Potatoes .....	1919		23.3	29.0	19.4	26.8	26.9	22.7
Tomatoes.....	1920		35.0	28.9	31.8	26.3	34.0	19.9
Lima beans.....	1921		12.6	19.8	36.6	31.9	43.3	49.2
Cucumbers .....	1922		5.5	9.4	8.9	10.4	9.7	13.1
<i>Average</i> .....			27.0	31.2	33.3	33.5	36.8	35.8

\* Omitted from average.

† Per cent nitrogen not determined; estimated from 1915 and 1920 crops at 0.18 per cent.

‡ Per cent nitrogen not determined; estimated from 1922 crop at 0.11 per cent.

the average amount returned for the first and second 5-year periods. The average amount for the third 5-year period is slightly higher, due chiefly to the larger amount returned in the corn crop of 1918.

Of the crops in this rotation corn required the most nitrogen and the cucumbers the least. The 5-year averages showed an increase for the limed plots over the check plots. The lima beans for 1921 showed the largest increase, the 2-ton application of calcium limestone gave a yield of 49.2 pounds as against 12.6 pounds for the check. The corn, potatoes, tomatoes and cucumbers did not show large increases for the lime treatment, though in nearly all cases there was some increase. Taking the averages, the yields with the 1- and 2-ton applications were very nearly the same for the three periods and were only a little higher than the yields with the 1-ton application. By the same standard the calcium and magnesian limestone are about evenly balanced.

#### ROTATION 4 (PLOTS 42-48)

The yields of dry matter for this rotation are shown in table 7. This was originally planned as a forage crop rotation. With the exception of the year when corn comes in, two crops have been grown each year, one of which has been a legume. In some cases soybeans have been substituted for cowpeas, and in 1922 rye took the place of oats and peas. In most cases the corn did not show very large increases with the lime treatments. On the other hand fair increases were generally obtained where a legume crop appears. The winter vetch and cowpeas (or soybeans) show marked response to the lime. For the corn, oats and peas and millet, the 1- and 2-ton applications of limestone have not greatly increased the yields over the  $\frac{1}{2}$ -ton application.

With two or three exceptions, notably the 2-ton magnesian-limestone treatment, the corn showed fair gains for the lime treatments. For the other crops of the rotation distinct increases are noted in the majority of cases. For example, in 1921 the rye and cowpeas on the no-lime plot yielded 5070 pounds of hay, whereas with 1 ton of calcium limestone the yield was 8208 pounds and with 1 ton of magnesian limestone, 9600 pounds. Again in 1922 the oats and peas and cowpeas on the no-lime plot yielded 3920 pounds while with one ton of both forms of limestone the yield was over 6000 pounds of hay.

In most cases the 2-ton application of calcium limestone gave larger yields than one ton of either, and in 12 cases out of 15 it gave larger yields than the two tons of magnesian limestone.

The 15-year averages show a slight advantage in favor of the magnesian limestone when used in  $\frac{1}{2}$ - and 1-ton amounts, but with the 2-ton application there is some indication of injury.

The results taken as a whole indicate that magnesian limestone favors nitrogen fixation rather more than does calcium limestone, although as already pointed out there were a few cases where the 2-ton application undoubtedly resulted in some injury to the crop.

TABLE 7  
*Crop yields in rotation 4 (acre basis)*

YEAR	NO LIME	$\frac{1}{2}$ TON		1 TON		2 TONS	
		Ca limestone	Mg limestone	Ca limestone	Mg limestone	Ca limestone	Mg limestone
Corn (grain and stover)							
	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
1908	4218	4232	5203	4645	4942	5345	3827
1913	4025	4600	3975	4825	4525	5325	4200
1918	5025	5484	5340	5365	5030	5723	5054
Average.....	4423	4772	4839	4945	4832	5464	4360
Oats and peas—millet							
	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
1909	3136	3240	3689	2750	3797	3322	3659
1914	1920	2680	2260	2820	2360	2960	2220
1919	2760	4680	4040	4800	4000	4920	3920
Average.....	2605	3533	3330	3457	3386	3734	3266
Winter vetch and rape							
	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
1910	2900	4300	4900	5250	3350	5650	5650
1915	1920	2680	3840	3520	4960	4280	5420
1920	4200	4008	3600	3372	4820	4560	4580
Average.....	3007	3663	4113	4047	4377	4830	5217
Rye and cowpeas							
	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
1911	4950	5500	4700	5850	5000	5550	5100
1916	4180	4900	5240	5320	5740	6000	4780
1921	5070	7560	8880	8208	9600	9172	8620
Average.....	4733	5987	6273	6459	6780	6907	6167
Oats and peas—cowpeas							
	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
1912	3050	3675	3425	3900	3475	3500	3475
1917	3280	3840	4480	4420	4420	4720	4420
1922	3920	5920	6280	6080	6480	7280	6400
Average.....	3417	4478	4728	4800	4792	5167	4765
GENERAL AVERAGE...	3637	4486	4656	4741	4833	5220	4755

*The yield of nitrogen*

This rotation has yielded much more nitrogen than any of the three preceding rotations. This reflects, in a striking manner, the influence of the legumes. As shown in table 8, the yields for the vetch and rape in 1910 ranged from 66.6 pounds for the check plot to 137.1 and 147.7 pounds, respectively,

TABLE 8  
*Yield of nitrogen per acre in different crops in rotation 4*

CROP	YEAR	NO LIME	½ TON		1 TON		2 TONS	
			Ca lime- stone	Mg lime- stone	Ca lime- stone	Mg lime- stone	Ca lime- stone	Mg lime- stone
First 5-year period, 1908-1912								
Corn.....	1908	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
Oats and peas, millet.....	1909	37.6	39.8	47.9	42.0	46.2	53.9	37.7
Rape, vetch.....	1910	54.1	57.1	68.2	50.6	71.3	59.4	61.0
Rape, vetch.....	1910	66.6	90.7	125.6	129.8	136.6	137.1	147.7
Rye, cowpeas.....	1911	90.4	106.2	77.9	116.6	82.8	98.6	87.5
Oats and peas, cowpeas.....	1912	64.5	90.1	75.8	103.0	87.3	84.1	90.6
Average.....		62.6	76.8	79.1	88.4	84.8	86.6	84.9
Second 5-year period, 1913-1917								
Corn.....	1913	37.0	45.3	39.8	52.1	43.0	55.4	44.8
Oats and peas, millet.....	1914	29.9	47.5	40.1	50.4	39.4	54.1	42.9
Vetch, rape.....	1915	23.8	47.1	79.3	71.7	109.8	90.2	116.7
Rye, cowpeas.....	1916	52.0	57.8	85.7	69.5	95.9	95.8	78.0
Oats and peas, cowpeas.....	1917	59.7	77.4	104.8	95.7	104.9	121.0	119.4
Average.....		40.5	55.0	69.9	67.9	78.6	83.3	80.3
Third 5-year period, 1918-1922								
Corn.....	1918	59.4	61.6	63.0	56.6	58.3	66.7	56.5
Oats and peas, millet.....	1919	36.5	62.5	55.1	70.6	54.7	72.0	57.1
Rye and vetch, rape.....	1920	32.9	45.6	47.8	41.1	74.2	55.2	69.6
Rye and vetch, soybeans.....	1921	61.9	120.1	156.6	175.7	145.8	163.7	172.5
Rye and soybeans.....	1922	50.9	82.0	87.6	84.6	115.1	120.7	117.3
Average.....		48.1	74.4	82.0	85.7	89.6	95.7	94.6

for the 2-ton applications of the calcium and magnesian limestone. In 1915 the same crops gave a yield ranging from 23.8 pounds for the check plot to 116.7 pounds for the 2-ton application of magnesian limestone. In 1920 when rye was seeded with the vetch the yield of nitrogen was not so high. The highest yields for the entire period were obtained with rye and vetch, and soybeans in 1921. In this case the check plot yielded 61.9 pounds and the largest yield was 175.7 pounds with one ton of calcium limestone.

With very few exceptions the yield was less on the check plot than on the lime treated plots. In the majority of cases the 1-ton application yielded more than the  $\frac{1}{2}$ -ton application. Judged by the 5-year averages, the 2-ton application shows very little advantage over the one ton. By the same standard the magnesian limestone shows a slight advantage over the calcium limestone in five out of nine chances.

The applications of commercial nitrogen for this rotation have been very light and it is therefore evident that the legume crops have drawn their supply of nitrogen largely from the atmosphere, otherwise the yields could not have been maintained as they have for a period of 15 years. Furthermore the nitrogen determinations on samples of soil from these plots indicate that the soil is not losing in its content of nitrogen.

#### INFLUENCE OF LIME ON THE PERCENTAGE OF NITROGEN IN THE CROP

It is well known that under certain conditions the nitrogen content of plants may be considerably modified. An increase in the amount of readily available nitrogen frequently results in an increase in the percentage of nitrogen in the crop.

In pot experiments with sand cultures it has been possible to increase the percentage of nitrogen in the dry matter from about 1 per cent under normal conditions to nearly  $3\frac{1}{2}$  per cent, by the use of large amounts of nitrate of soda (6). Corn and other non-legume crops grown after a legume cover crop will frequently show a higher percentage of nitrogen in the dry matter than when the same crop is grown after a non-legume green manure crop.

On the other hand nitrogen starvation may also result in a plant with a per cent of nitrogen higher than normal. It has been shown that in the majority of cases, legume crops grown on limed land, recover a larger amount of nitrogen per acre than the same crops grown on unlimed acid land. This may be due to an increase in the yield of dry matter or to a higher percentage of nitrogen in the dry matter, or both.

Apparently the cause of the higher percentage of nitrogen in the dry matter from limed plots, as contrasted with that from unlimed plots, has not been fully investigated. It may be due in part to the fact that frequently there is a greater abundance of weeds and grasses on the unlimed than on the limed plots. In this connection Hall (2) says: "Again on examining the composition of the herbage, it was seen that on the same three plots which gave an increase of crop, the lime has brought about a great increase in the proportion of leguminous plants. On plot 6 it has risen from 11 to 20 per cent, on plot 7 from 22 to 42 per cent and on plot 15 from 3 to 35 per cent." Hall believes that the development of the legume plant is largely dependent on a supply of potash, and further points out that these three plots have received potash every year for some time and consequently there was a large reserve of potash in the soil. The lime he believes, released this potash and therefore acted as an

application of potash. In other words, according to Hall's view, the lime acts only where there is a residue of potash.

Hall's line of reasoning is not entirely borne out by the work at the New Jersey Station. Here even with liberal annual applications of soluble potash, volunteer legumes do not come in to any extent if lime is withheld. On the other hand when lime is applied under these conditions, it is practically impossible to prevent the coming in of volunteer legumes, the clovers especially. It would seem that the differences noted may be reconciled by the fact that the Rothamsted soils are well supplied with calcium carbonate.

It would appear, that the percentage of nitrogen in the dry matter may be very much influenced by the amount of available nitrogen at the disposal of the growing crop. In the case of the higher percentage of nitrogen from limed plots where the crop is strictly a legume, as in the case of soybeans grown for seed, the lime undoubtedly favors those organisms which aid the plant in getting nitrogen from the air, and the plant thus assisted, becomes a more efficient nitrogen accumulator. In confirmation of this view, counts have shown far more root nodules on soybean plants from limed than from unlimed plots (5); likewise the plants from the limed plots were much larger and healthier in appearance than those from the unlimed plots. This could account for the higher percentage of nitrogen in the immediate crop and might also mean more available nitrogen for the succeeding crop whether it be a legume or a non-legume.

Nitrogen determinations have been made for practically all of the crops in the four rotations for the 15-year period. These data are given in tables 9 and 10 for the crops of rotations 1 and 4 which are fairly representative. They have been further shortened by averaging the figures for the three amounts of limestone in each case; i.e., the calcium limestone is represented by averaging the figures for the  $\frac{1}{2}$ -, 1-, and 2-ton applications and the figures representing the magnesian limestone were obtained in a similar manner.

A study of these two tables will show that in the majority of cases crops from the limed plots showed a higher percentage of nitrogen than those from the unlimed plots. A closer examination however, shows that the legume crops are largely responsible for throwing the balance in favor of the lime treated plots. In the case of the non-legume crops alone, the figures are frequently reversed. The legume crops on the other hand frequently show a striking increase in percentage of nitrogen for the limed plots over the unlimed plots. For example, cowpeas in 1916 in rotation 4 showed 1.47 per cent nitrogen with no lime and 2.33 per cent with magnesian limestone; the same crop in 1922 shows 1.51 per cent nitrogen with no lime and 2.48 per cent for the magnesian limestone, the difference being nearly 1 per cent in each case.

For a given crop the percentage of nitrogen does not vary greatly for the two forms of limestone, but in the majority of cases there is a slight difference in favor of the magnesian limestone. This is shown by the figures in the right hand column of the tables.



TABLE 9  
*Nitrogen in the crops of rotation 1*

LIME TREATMENT	CORN—FIRST YEAR		OATS—SECOND YEAR		WHEAT—THIRD YEAR		TIMOTHY AND CLOVER —FOURTH YEAR		TIMOTHY AND CLOVER —FIFTH YEAR	
	Grain	Stover	Grain	Straw	Grain	Straw	1st cutting	2d cutting	1st cutting	2d cutting
	per cent	per cent	per cent	per cent	per cent	per cent				
No lime	{ 1908-1912	1.31	0.65	2.20	0.86	1.91	0.45	2.12†	0.97†	0.85
	{ 1913-1917	1.213	0.573	2.024	0.731	1.968	0.295	0.866	2.161	0.760
	{ 1918-1922	1.469	0.712	2.224	0.526	1.712*	0.870*	0.874	0.945	1.074
Ca limestone	{ 1908-1912	1.32	0.77	2.26	0.85	1.78	0.44	2.14†	0.82†	0.86
	{ 1913-1917	1.335	0.744	2.196	0.808	2.003	0.311	1.174	2.596	0.836
	{ 1918-1922	1.488	0.789	2.455	0.773	1.732*	0.538*	0.837	1.126	1.700
Mg limestone	{ 1908-1912	1.37	0.85	2.33	0.92	1.84	0.43	2.26†	0.91†	0.90
	{ 1913-1917	1.377	0.770	2.246	0.844	1.994	0.321	1.122	2.648	0.819
	{ 1918-1920	1.541	0.848	2.443	0.882	1.792*	0.603*	0.798	1.117	1.544

\* Barley.

† Oats (grain). ‡ Oats (straw).

TABLE 10  
*Nitrogen in the crops of rotation 4*

LIME TREATMENT	COIN—FIRST YEAR		SECOND YEAR		THIRD YEAR		FOURTH YEAR		FIFTH YEAR	
	Grain	Stover	Oats and peas	Millet	Vetch	Rape	Rye	Cowpeas	Oats and peas	Cowpeas
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
No lime	1.30	0.64	2.01	1.28	3.11	1.54	1.47	2.33	1.98	2.35
	1.361	0.721	1.658	1.186	3.216	0.981	1.049	1.470	1.293	2.785
	1.544	0.906	1.386	1.160	0.756*	0.818	1.141*	1.288†	1.053†	1.506
Ca limestone	1.40	0.72	2.26	1.26	3.12	1.51	1.42	2.72	2.29	2.83
	1.420	0.837	1.893	1.379	3.335	1.064	1.095	1.653	1.823	2.968
	1.423	0.916	1.415	1.462	1.185*	1.216	1.850*	1.818†	0.718†	2.313
Mg limestone	1.44	0.72	2.38	1.11	3.39	1.49	1.23	2.51	2.23	2.84
	1.414	0.794	1.841	1.432	2.916	1.133	1.020	2.329	2.054	3.274
	1.500	0.917	1.402	1.379	1.516*	1.277	2.038*	1.457†	0.849†	2.484

\* Rye and vetch. † Soybeans. ‡ Rye.

TABLE 11  
*Lime requirement and nitrogen content of all soils at the close of each period  
 (and pH values for 1922)*

PLOT NUMBER	TREATMENT PER ACRE	LIME (CaO) REQUIREMENT PER 2,000,000 POUNDS OF SOIL			NITROGEN CONTENT				REACTION 1922
		1913*	1918†	1922‡	1913	1918	1922		
							Soil 1		
Rotation 1.—General farm crops									
21	No lime	1200	1200	1500	0.077	0.0794	0.082	0.059	5.2
22	Ca limestone:								
	0.5	1000	600	800	0.075	0.0798	0.082	0.052	6.3
23	1.0	600	400	400	0.075	0.0791	0.084	0.051	6.4
24	2.0	000	Alk.	Alk.	0.081	0.0901	0.092	0.061	6.8
	Mg limestone:								
25	0.5	600	400	1000	0.091	0.0959	0.095	0.062	5.7
26	1.0	700	400	600	0.093	0.1015	0.108	0.074	6.4
27	2.0	000	Alk.	Alk.	0.092	0.1010	0.108	0.071	7.0
Rotation 2.—General farm crops and potatoes									
28	No lime	800	1400	1400	0.094	0.0994	0.102	0.067	5.6
	Ca limestone:								
29	0.5	800	1000	900	0.097	0.1019	0.101	0.065	5.6
30	1.0	600	400	300	0.094	0.0931	0.097	0.059	6.4
31	2.0	100	Alk.	100	0.098	0.1041	0.101	0.058	6.8
	Mg limestone:								
32	0.5	700	400	600	0.093	0.1025	0.100	0.058	6.4
33	1.0	400	Alk.	300	0.091	0.0954	0.097	0.054	6.7
34	2.0	300	200	100	0.089	0.0966	0.100	0.049	6.9
Rotation 3.—Corn, potatoes, market garden crops									
35	No lime	1100	1200	1200	0.093	0.0921	0.093	0.062	5.4
	Ca limestone:								
36	0.5	800	1000	900	0.094	0.0912	0.093	0.059	5.5
37	1.0	600	800	600	0.081	0.0837	0.087	0.055	6.3
38	2.0	400	Alk.	300	0.075	0.0808	0.083	0.055	6.8
	Mg limestone:								
39	0.5	1100	1000	600	0.077	0.0800	0.080	0.050	6.2
40	1.0	700	400	600	0.080	0.0837	0.082	0.060	6.4
41	2.0	500	400	200	0.082	0.0800	0.081	0.059	6.9

\* Limestone applied in spring 1908.

† Limestone applied in spring 1913.

‡ Limestone applied in spring 1918.

TABLE 11.—Continued

PLOT NUMBER	TREATMENT PER ACRE	LIME (CaO) REQUIREMENT PER 2,000,000 POUNDS OF SOIL			NITROGEN CONTENT			REACTION 1922
		1913*	1918†	1922‡	1913	1918	1922	
							Soil 1	
Rotation 4.—Forage crops								
	tons	lbs.	lbs.	lbs.	per cent	per cent	per cent	pH
42	No lime	1200	1400	1200	0.084	0.0899	0.090	5.2
	Ca limestone:							
43	0.5	1100	1200	1000	0.095	0.0998	0.101	5.5
44	1.0	700	800	600	0.099	0.1012	0.102	6.3
45	2.0	600	400	200	0.089	0.0864	0.084	6.7
	Mg limestone:							
46	0.5	1100	1200	600	0.079	0.0773	0.077	6.3
47	1.0	500	400	400	0.067	0.0679	0.069	6.4
48	2.0	300	Alk.	200	0.098§	0.0652	0.063	6.8
AVERAGE.....					0.087	0.0893	0.091	

§ Probably an error in determination; 1914 sample gives 0.070 per cent N.

The rye and vetch crop in 1920 in rotation 4 supplies a good illustration of the value of lime when a legume crop is combined with a non-legume. Here the no-lime plot where the vetch did poorly showed 0.756 per cent nitrogen while the plots that received magnesian limestone showed a percentage just twice as great. Besides a large increase in dry matter already referred to, the lime-treated plots yielded a feeding material much richer in protein than the unlimed.

#### THE INFLUENCE OF THE LIME ON THE LIME REQUIREMENT AND NITROGEN CONTENT OF THE SOIL

All of these soils have been analyzed for lime requirement and total nitrogen at the close of each 5-year period. The figures for the first two periods have been published (3, 4). They are given here again for comparison with the data for the third 5-year period (table 11). The hydrogen-ion concentration in terms of pH are reported for the 1922 samples.

In collecting samples, a number of borings were taken from each plot to a depth of 6½ inches and from these a composite sample was made. The portion of the dry sample not passing a 2-mm. sieve was discarded. Nitrogen determinations were made by the Kjeldahl method and lime requirement by the Veitch method.

### *Lime requirement*

The figures for lime requirement show a general decrease with increase of lime application, the 2-ton application leaving the soil alkaline or nearly so after 5 years. With one exception the no-lime plots showed but little change since the first sampling in 1913 and the requirement ranged between 1000 and 1500 pounds CaO per acre. With a few exceptions, the plots which have received one-half ton of limestone per acre showed a requirement close to 1000 pounds and those that have received one ton per acre, a requirement ranging in most cases between 300 and 600 pounds.

The magnesian limestone appears to have a slight advantage in the matter of satisfying the lime requirement.

The hydrogen-ion determinations are in general accord with the lime requirement determinations. For example, the four no-lime plots showed a range of pH 5.2 to pH 5.6 and the plots which received two tons of limestone per acre a range of pH 6.7 to pH 7.0. From these figures it would appear that in many cases at least, the pH values might be used as a measure of the lime requirement.

### *Percentage of nitrogen in the soil*

Figures showing percentage of nitrogen in the soil are quite consistent for the three periods. It is gratifying indeed to find that the figures are in most cases slightly higher for the third period than for the two preceding periods. This is confirmatory evidence that under the different systems of cropping the soil is not being depleted of its stores of nitrogen and organic matter. Furthermore, this result is being accomplished without the use of any farm manure and, excepting the market garden rotation, with a minimum of commercial nitrogen.

It would appear from the percentage of nitrogen shown for plots 21, 22, 23, and 46, 47, 48, that the soil of these plots is naturally not so good as the soil constituting the remainder of the plots. The percentages of nitrogen in the subsoil seem to confirm this view. Taking all rotations for the entire period there is a slight increase in the percentage of nitrogen as shown by the general average for 1922, in comparison with the averages at the two preceding sampling periods. It is highly probable that these soils would have decreased in percentage of nitrogen rather than increased, had legume crops been entirely omitted from the rotations.

Taking the results for all of the rotations, it may be said that there is no definite indication that the lime has tended to cause an unusually rapid disappearance of the nitrogen from the soil.

### SUMMARY

1. An experiment involving the use of magnesian and non-magnesian limestone, in three different amounts, as compared with a check plot has been carried out for a period of 15 years, in connection with four different 5-year crop rotations.

2. The soil is a Sassafras loam, gravelly phase, and previous to the experiment had not been limed for many years.

3. In each of the four rotations legume crops were introduced to some extent, either as one of the main crops or as a green manure crop between the main crops.

4. Mineral fertilizers (acid phosphate and muriate of potash), have been used at the rate of 300-400 pounds per acre for the former and 100-200 pounds for the latter. Commercial nitrogenous fertilizers in amounts equivalent to 160-200 pounds per acre of nitrate of soda have been used. No farm manure has been used during the entire 15 years.

5. With few exceptions the lime-treated plots have shown substantial increases in crop yield over the check plots. Of the crops used in the various rotations the legumes have shown a greater response to the lime treatments than the non-legumes, though the latter have usually shown some response.

6. In most cases the 1-ton application has given some increase over the  $\frac{1}{2}$ -ton application but, in a number of cases at least, this increase is not sufficient to justify the additional expense. In the majority of cases the 2-ton application gave but slight increase in yield over the 1-ton application thus indicating that the 2-ton application is excessive from the standpoint of economy.

7. In a few cases there was indication of crop injury from the use of 4000 pounds of magnesian limestone per acre. Aside from this the two forms of limestone gave results that are quite similar, though taking the records for the entire period, there is a slight difference in favor of the magnesian limestone.

8. In the matter of the amount of nitrogen recovered from the crop, the difference between the unlimed and limed plots is more striking than the differences in the case of the crop yields, but here as with the crop yields, the 2-ton application gave very little increase over the 1-ton application.

In this case, also, the magnesian limestone seemed to show a slight advantage over the other form.

9. For the legume crops especially, the limed plots showed a higher percentage of nitrogen in the dry matter than the non-legume crops. In some cases the difference was very pronounced. This clearly indicates an improvement in the quality of the crop as well as an increase in the quantity.

10. In the case of mixed crops, such as mixed hay, it is suggested that this increase in percentage of nitrogen may have been due in part to the fact that the lime stimulates the growth of legume plants to the detriment of non-legumes.

In the case of strictly legume crops it is suggested that the increase in nitrogen content may have been due to the stimulating effect of the lime on the organisms which aid the plant in getting nitrogen from the air.

11. There is evidence that the magnesian limestone favors nitrogen fixation rather more than calcium limestone.

12. With the increase in the amount of lime applied there is a decrease in the lime requirement of the soil as determined by the Veitch method. The

2-ton application left the soil near the neutral point at the end of each 5-year period. With one ton the requirement is in most cases between 300 and 600 pounds and with one-half ton about 600 to 1200 pounds per acre. In most cases the check plots indicate a requirement of about 1200 to 1500 pounds per acre.

13. The hydrogen-ion concentration decreased gradually as the lime applications were increased, and the work indicates that this method may, in some circumstances, be used for the determination of the lime requirement.

14. The nitrogen content of the soil has remained fairly constant during the last 10 years of the period with a slight upward tendency. This stands as evidence that under the systems of cropping practiced in this experiment the soil is not being depleted of nitrogen and organic matter. Neither are the yields decreasing. Undoubtedly the legume crops have been a factor in maintaining the fertility of the soil.

15. In general there is no definite indication that lime has tended to cause an abnormally rapid disappearance of nitrogen and organic matter from the soil.

16. The results of this experiment indicate that it is not necessary to fully satisfy the lime requirement of the soil as commonly expressed in order to get good results with most farm crops.

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## INFLUENCE OF SOIL CONDITION ON BACTERIAL LIFE AND CHANGES IN SOIL SUBSTANCE: II. ABILITY OF SOIL TO BREAK DOWN MANNITE

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In articles already published (1) the author has described preliminary investigations of the ability of soil to break down mannite. These investigations, which were generally qualitative, showed that the ability of the soil to break down this substance, as well as an entire series of other organic substances, depends largely on their chemical conditions.

The close relationship between the mannite-fermentation power of the soil and its content of certain substances (lime and phosphoric acid) made it seem desirable to find a method by which this power might be measured quantitatively. The efforts along that line and the results of a series of special investigations of the requirements for mannite decomposition and its relation to the condition of the soil will be discussed in the present paper.

In hitherto unpublished investigations on the ability of a series of various soils to bind nitrogen from the air, made by mixing the soil portion in question with mannite (2 per cent) and letting them stand for a certain period at a temperature of 25°C., it was observed that much mannite remained in some of the soils. Upon drying some of the soils considerable quantities crystallized on the surface of the soil, while on others only a little crystallization was seen and on others again, none at all. It was evident that the ability of the various soils to break down the mannite added varied greatly. The question now to be answered was how best to determine the amount of unchanged mannite remaining in the soil.

For many reasons direct mannite determination was difficult. On considering the question more carefully it seemed correct to assume that a determination of the entire quantity of soluble organic matter coming from mannite and remaining in the soil would better express the total change of matter and so an attempt was made to determine this content in a way similar to that in which the organic matter in drinking water is usually determined.

The method used in the first step of the investigation was as follows:

An average sample of about 5 gm. was drawn from moist soil containing mannite. This portion was air-dried,<sup>1</sup> weighed and placed in a beaker containing 100 cc. distilled water. After standing for 2 hours with occasional shaking, the mixture was filtered and a certain

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<sup>1</sup> Air-drying causes no definite diminution of the content of organic matter.



quantity, as a rule 10 cc., of the clear filtrate was placed in a beaker. To this was added 50 cc. of 0.02 *N* potassium permanganate solution and 3 cc. dilute sulfuric acid (6:100). After heating over a boiling water-bath for 10 minutes, 50 cc. of 0.02 *N* oxalic acid solution was added to the mixture. After continued heating until this was again clear, it was titrated back with 0.02 *N* potassium permanganate solution. The amount of this solution used expresses the amount of soluble organic matter in the soil.

In the course of the investigation this method has been somewhat modified

In the preliminary experiments, the mixture was heated over small water-baths, each containing a single beaker, and as a rule the results of the replicate analyses agreed. However, as the investigations soon became more extensive, the mixture was heated over larger water-baths where there was room for 8 beakers. Now the results of the replicate analyses were often found to disagree. A special investigation showed that this was due to the fact that the temperature attained in the beakers placed in the large water-bath was too low to permit complete oxidation of the organic substances in 10 minutes, and that the temperature varied in the different parts of the water-bath. The degree of heat in the glasses varied between 55–70°C. while in the small water-bath the solution was heated to a temperature of about 80°C. By using a longer period, (20 minutes), and by immersing the beakers directly in the boiling water in the water-bath instead of placing them over the holes the temperature of the solution soon rose to about 80° and the results of the analyses agreed.

The influence of the soil-water content on the power to break down mannite must be established.

In making these investigations a light gray sandy soil and two loamy soils were used. Whereas one portion of sandy soil and one of the loamy soils were unmixed the other portion of sandy soil and the other loamy soil were mixed with carbonate of lime and di-basic potassium phosphate. Tests were made with the application of 4 different amounts of water corresponding to 40, 60, 80 and 100 per cent of the total water-holding capacity.

The water-holding capacity was determined by the following method:

Twenty grams of soil was thoroughly stirred with a surplus of water in a porcelain bowl. The thick soil-gruel was then poured into a funnel tipped with a small moistened filter about 1 cm. long. The funnel was covered with a sheet of glass, and after all dripping had ceased usually on the following day, 5 gm. of the water-soaked soil was weighed into a weighing glass preparatory to determining its oven-dry weight.

In determining the content of water in the air-dry soil, which content must be reckoned with in measuring the water, 5 gm. of soil were weighed out on a watch glass. This, and the water-saturated soil-portion were placed in a desiccator at 100°C. The water-holding capacity is given in per cent of oven-dry soil.

After determining the water-holding capacity, 8 portions of 100 gm. air-dry soil were weighed out into Petri dishes (2 x 10 cm.). This soil was stirred until it formed a homogenous thick layer, loose in structure, at the bottom of each dish. An amount of distilled water was pipetted into each dish corresponding to the desired percentage of the full water-holding capacity of the soil. The water should be dropped—and this is particularly to be recommended in the case of clay soils,—along the edge of the dish, so that it may be absorbed by the soil through capillary action without disturbing its loose structure. In using the largest quantity of water, corresponding to 100 per cent of the water-holding capacity, the loose structure cannot be maintained and clay soils, in particular, tend to become "fluid." This checks the air-supply very materially and as a new factor is thereby introduced into the investigation it becomes impossible by this method to obtain an absolutely true expression for the influence of the water-content on the course of decomposition. The dishes were

covered and kept at a temperature of 25°C. Every fifth day a sample was drawn from each separate dish and the amount of water evaporated during the period replaced by dropping in distilled water.

Results of these investigations may be seen in table 1.

The water-content of the soil determines primarily not only the speed, but also the regularity at which mannite decomposition takes place. The content of water which, under the given conditions, seems most favorable to mannite

TABLE 1  
*Influence of the soils content of water on decomposition of mannite*

MOISTURE IN TERMS OF WATER-HOLDING CAPACITY	CONTENT OF ORGANIC MATTER AFTER VARIOUS DECOMPOSITION PERIODS*					
	5 days	10 days	15 days	20 days	25 days	30 days
Light gray sandy soil (water-holding capacity 23.5 per cent)						
<i>per cent</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>
40	32.6	28.0	29.1	30.7	31.3	24.3
60	33.3	31.1	31.0	24.6	17.1	13.2
80	32.1	29.2	29.9	20.2	9.4	3.5
100	28.9	21.8	18.7	15.0	12.2	11.1
Same soil treated with 0.5 gm. $\text{CaCO}_3$ + 0.1 gm. $\text{K}_2\text{HPO}_4$						
40	34.5	28.9	30.1	29.8	28.4	25.5
60	34.1	28.4	1.7			
80	34.1	1.0				
100	32.3	7.5	0.7			
Loamy soil A (water-holding capacity 38.9 per cent)						
40	23.3	26.8	26.3	25.3	17.8	10.9
60	23.7	24.4	20.2	10.4	1.4	0.5
80	24.3	20.5	14.5	3.5	0.4	
100	24.7	15.0	17.0	3.1	0.5	
Loamy soil B treated with 0.5 gm. $\text{CaCO}_3$ + 0.1 gm. $\text{K}_2\text{HPO}_4$ (water-holding capacity 28.5 per cent)						
40	31.4	21.0	17.1	10.5	1.0	
60	27.8	16.6	10.0	0.8		
80	29.9	1.0	0.6			
100	26.8	1.0	0.4			

\* Organic matter is expressed here and in other tables in cubic centimeters of 0.02 *N* potassium permanganate required per 0.5 gm. of dry soil for oxidation. These data are averages of duplicate determinations.

decomposition is that corresponding to about 80 per cent of the absolute water-holding capacity. In the following investigations an amount of water corresponding to 75 per cent of this capacity has been used. This content of water does not destroy the loose structure of the soil whereas soil, and particularly heavy soil, with a water content of 80 per cent tends to become "fluid" and soggy.

Methodical investigations and experience have given us the following complete method for determining the power of mineral soils to break down mannite.

From the soil samples to be tested, an amount of soil corresponding to 100 gm. air-dry soil was weighed out in tumblers 9.5 cm. in diameter and 6.5 cm. deep and mixed with 2 gm. mannite. Distilled water, corresponding to 75 per cent of the water-holding capacity of the soil was added. In investigations of air-dry soils with a somewhat large water-holding capacity, the mannite may be added dissolved in water. When the water has been added, the tumbler must stand undisturbed for 2 to 3 hours so that the water can penetrate the entire soil-portion. The soil is now carefully stirred with the glass spatula and arranged in a homogenous, thick, lightly-resting layer at the bottom of each tumbler. The tumblers were weighed and kept at a temperature of 25°C. The following day the soil was stirred and after 5 days the water lost by evaporation was replaced. When this had thoroughly penetrated the soil, the mass was stirred, (care being taken not to destroy its loose structure) and about 5 gm. were weighed out for air-drying. The tumbler was again weighed and replaced in the incubator. Samples were drawn 5 days later, and then the process was repeated.

The air-dry samples were weighed again, placed in a glass beaker containing 100 cc. of distilled water, left to stand for two hours with occasional shaking, and filtered through filter paper. To 10 cc. of the clear filtrate was added 50 cc. of 0.02 *N* potassium permanganate solution and 3 cc. 6-per cent sulfuric acid.<sup>2</sup> The beaker was then placed in boiling water and allowed to remain for 20 minutes (the 10 minutes used hitherto was found too short). Fifty cubic centimeters of 0.02 *N* oxalic acid solution was added and when, under constant heating, the liquid became as clear as water (usually, after a few minutes), it was titrated with a 0.02 *N* potassium permanganate solution. The beakers must remain in the water bath until they can be titrated. The amount of potassium permanganate solution used expresses the content of organic matter. The amount of potassium permanganate solution is expressed per  $\frac{1}{2}$  gm. of air-dried soil.

#### INVESTIGATIONS ON THE ABILITY OF VARIOUS SOILS TO BREAK DOWN MANNITE

The first point to be made clear in the further investigations on this subject was the limit within which the ability of different soils to break down mannite varied.

In these investigations a series of field soils which had been sent in to the laboratory with another object in view were used.

Samples 17, 21, 22, 25, 26, 27, 35, 37, 68 and 69 were drawn from unfertilized plots in local field experiments made in 1916 or 1917 in which the addition of superphosphates caused a greater or less positive reaction. However the lack of phosphoric acid in the soils in question cannot be positively established on the basis of results from this one year. Sample 43 was drawn from plots in a fertilization experiment at Askov Experiment Station which have remained unfertilized for many years, and whose soil is now greatly in need of phosphoric acid (cf. p. 350). Sample 59, from Oelstykke, should also be considered as needing phosphoric acid (cf. p. 351). Samples 10 and 42 are drawn from experimental plots which have been treated with a large quantity of superphosphates for many years, and which cannot be assumed to lack phos-

<sup>2</sup> In order to be certain that the sulfuric acid does not contain organic substances, it is desirable to boil the concentrated acid to be used for a couple of hours.

phoric acid. All the above-named soils are indicated in table 2 by an asterisk (\*) in the soil-number column. In the case of the other soils it is not known whether or not they lack phosphoric acid.

Before being used, the soil samples were air-dried and sifted through a 1.5-mm. round-hole sieve. Very fine subdivision of the soil was avoided.

In investigations of this nature, the use of air-dried samples, *particularly when they are used shortly after being dried* can hardly occasion scruples in regard to principle,<sup>3</sup> for it must be remembered that in nature the upper layer of soil is often in that condition, and sometimes for long periods of time. The fact that air-drying, as various scientists have demonstrated, can to some extent change the chemical and microbiological condition of the soil is another matter. Therefore in comparative experiments in decomposition of matter either moist or dry soil should be used in every case.

In order to investigate the extent to which the differences between the power of various soils to decompose mannite depend on chemical or microbiological factors, both "*inoculated*" and "*uninoculated*" cultures have been set aside in accordance with the author's former principles of investigation (1).

A mannite solution admixed with soil and strongly fermented was used for inoculating. The inoculation was made with a platinum loop, using four loops of liquid culture which were then well mixed into the soil.

The grade of decomposition was determined every five days until 30 days after the experiment was begun, if decomposition had not ceased hitherto. Check determinations of soil samples without admixture of mannite have not been made in every case, but a series of investigations of different soils has shown that the content of water-soluble organic matter per  $\frac{1}{2}$ -gram air-dry soil, is only by exception greater than the amount which 1-2 cc. of .02 *N* potassium permanganate solution will oxidize. When the content of such organic matter per  $\frac{1}{2}$ -gram in soils mixed with mannite is within the oxidizing capacity of 3-4 cc. of potassium permanganate solution, decomposition is considered complete.

In table 2, the soil samples are arranged in groups according to their physical condition (sandy soils and loamy soils) and within these groups again according to their reaction. The results show that the ability of soils to break down mannite varied considerably. Soil samples 35, 44, 52, 62, 90, 93 and 95, for instance, showed great power to break down mannite for the mannite in the "*inoculated*" and "*uninoculated*" cultures almost entirely disappeared after 5 days. On the other hand, there were many soils which even after 25-30 days contained a large quantity of soluble organic matter. Samples 43 and 61 possessed the least power of decomposing mannite.

Table 2 gives no information in regard to the causes of the differences found. These can not be sought in the varying physical condition of the soils, for examples of slight and of great power to decompose mannite may be found both among light sandy soils and among heavy loamy soils. A closer connection

<sup>3</sup> See second paragraph of footnote 5.



10*	Tystofte	h., poor in humus	none	neut.	0	0.058	1.28	0.193	0.022	38.9	34.7	22.0	7.7	4.0	38.3	4.2	3.5	
11	Hakkebille-gaard	r. h., poor in humus	none	neut.	0	0.103	1.04	0.146	0.031	37.6	20.3	3.4			33.9	7.0	2.3	
12	Vigsted	h.	none	neut.	0	0.082	0.54	0.330	0.035	38.6	33.5	5.6	9.3	2.7	38.4	28.8	19.3	4.2
13	Skalsballe, Vigsted		none	neut.	0	0.067	0.62	0.321	0.029	39.2	36.3	35.5	31.8	23.5	39.6	36.6	34.7	30.7
14	Havdrup	l.	none	neut.	0	0.112	0.68	0.203	0.027	40.1	35.6	27.5	7.2	3.7	39.7	36.7	25.6	5.6
15	Voetfadegaard	poor in humus	none	neut.	0	0.134	2.22	0.218	0.027	39.5	36.6	34.4	27.0	17.0	36.5	31.8	26.6	10.8
16	Gjerlev	none	none	neut.	0	0.125	0.79	0.226	0.028	38.7	35.3	24.5	19.8	6.4	38.0	20.9	8.5	2.1
17*	Ny-Holme-gaard	l., r. poor in humus	v. sl.	neut.	0	0.070	0.76	0.244	0.007	31.8	18.5	11.9	2.1		30.3	13.9	5.5	0.8
18	Frammerslev-gaard	l.	none	neut.	1	0.144	8.70	0.214	0.035	38.7	3.5				4.9			
19	Beilinge	l.	none	neut.	3	0.099	1.16	0.193	0.027	34.8	26.2	23.5	15.1	2.2	36.8	29.8	28.7	17.6
21*	Strøby	h.	none	neut.	3	0.062	0.26	0.125	0.023	33.9	22.8	22.1	13.8	7.3	1.2	33.9	23.8	22.3
22*	Olstrup	none	none	neut.	3	0.081	0.64	0.197		27.3	24.3	16.5	1.0		25.9	23.8	10.0	0.6
23	Bjærgager Skov-gaard	r. h., poor in humus	v. sl.	neut.	3	0.081	1.14	0.280	0.035	38.4	4.0				31.0	2.5		
24	D G. U. 6	none	none	neut.	4	0.083	1.10	0.210	0.019	38.0	28.8	28.8	7.22	4.15	9.10	0.34	3.32	3.27
25*	Forlev 1916	r. h.	none	neut.	4	0.065	0.30	0.170	0.031	34.5	22.6	16.6	8.7	5.7	1.4	37.6	19.1	20.4
26*	Forlev 1917	r. h.	none	neut.	4	0.051	0.22	0.197	0.027	39.1	25.4	22.7	24.3	19.7	13.2	38.1	24.2	22.0

\* Not included in the summary given in table 6. (See further page 11.)

† The development of azotobacter is expressed in figures within the scale 0-4. 0 indicates no azotobacter development, 4 a maximum development (vigorous, slimy, often folded membrane over the entire surface of the solution).

‡ 100 per cent = weight of air-dry soil.

§ Through an error first determined after 21 days.

\* See footnote to table 1. These data are also averages of duplicate determinations.

† Abbreviations used are: alk. = alkaline, f. = fine or finely, h. = heavy, l. = light, neut. = neutral, r. = rather, sl. = slightly, str. = strong v = very.



37*	Slangerup	rich in iron	v. sl.	sl. alk.	4	0.102	1.58	0.285	0.017	38.6	22.2	13.0	1.5		38.6	16.6	7.1	0.7
38	Akurejri, Is-land a.		sl.	sl. alk.	4	0.212	1.58	0.601	0.148	33.5	3.6				4.7			
39	Akurejri, Is-land b.		none	sl. alk.	4	0.095	2.20	0.560	0.117	—	—	—	—	—	4.1			
40	Brøndager	v. h.	none	sl. alk.	4	0.080	2.70	0.282	0.027	39.4	30.0	15.0	11.6		27.3	5.7		
42*	Ex-field, Royal Agr. Coll.	h., rich in humus	sl.	alk.	4	0.127	3.34	0.524	0.023	19.0	5.7				3.8			
43*	Askov, B 3 unfertilized 1919	r. rich in humus	v. sl.	alk.	4	0.046	0.26	0.356	0.014	39.8	37.6	36.7	37.5	35.7	35.4	40.4	38.1	36.8
44	Kastrup Mølle	r. rich in humus	str.	alk.	4	0.177	10.00	0.409	0.032	1.0					3.6	1.3		35.9
45	Horshunde	r. poor in humus	r. str.	alk.	4	0.101	1.11	0.653	0.015	20.7	3.2				3.7			30.3
46	Rønhoft	r. poor in humus	sl.	alk.	4	0.093	3.28	0.343	0.020	33.1	18.6	2.1			12.6	4.3		25.3
47	Tystofte	r. h.	r. str.	str. alk.	4	0.062	0.88	—	—	7.9	1.3				2.4	0.9		
48	Grønvang	r. rich in humus	str.	str. alk.	4	0.056	0.54	0.450	0.026	15.7	1.7				17.5	1.2		
49	D. C. U. 8	h.	str.	str. alk.	4	0.112	1.5	0.469	0.024	18.8	1.1				11.5	0.7		
50	Borris	l.	str.	str. alk.	4	0.097	0.46	0.302	0.016	36.3	7.8	2.1			29.5	1.2		
51	Petersdal	l. rich in humus	v. str.	str. alk.	4	0.110	6.62	0.510	0.028	32.3	17.4	5.3			22.8	3.8		
52	Kastrup	l.	str.	str. alk.	4	0.061	3.30	0.506	0.022	3.4					2.9			
53	Gjerrild Lou Lolland	r. poor in humus	v. str.	str. alk.	4	0.071	0.46	1.110	0.022	37.1	32.2	23.2	11.8	4.5	33.7	23.6	9.9	4.2
54	Baadesgaard, Sølsted	h.	sl.	str. alk.	4	0.084	2.15	0.749	0.027	17.2	3.1				7.7	3.2		
55	Tjøncheved, Allerslev	v. h.	sl.	str. alk.	4	0.092	2.24	0.628	0.020	32.2	5.9				17.8	3.4		



Soil	Kind	General condition of the soil	Acid effervescence	Reaction	Development of azotobacter	Phosphoric acid content		CaO	MgO	Content of organic matter after various decomposition periods %											
						P <sub>2</sub> O <sub>5</sub> soluble in m-phosphoric acid	P <sub>2</sub> O <sub>5</sub> per liter of CO <sub>2</sub> saturated water			Uninoculated					Inoculated						
										per cent	mgm.	per cent	cc. #	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
Sandy soils																					
6	D. G. U. 5	sandy soil	none	acid	0	0.048	0.68			34.8	30.8	25.8	22.5	22.0							
7	Tylstrup	sandy soil, v. f. grained	none	acid	0	0.117	1.16	0.023	0.011	36.7	37.3	34.0	22.8	9.4	2.0	36.5	38.0	35.1	24.5	9.9	2.7
8	V. Hassing	sandy soil, v. f. grained	none	acid	0	0.099	1.10	0.106	0.021	38.2	36.3	27.2	19.1	11.2		35.6	27.9	14.0	4.1		
9*	Ølstykke	sandy soil	none	acid	0	0.066	0.26	0.130	0.017	38.8	36.5	35.0	30.2	13.4	4.9	38.7	36.1	34.8	30.9	19.2	8.7
10	Lundgaard II	l. sandy soil	none	sl. acid	0	0.050	0.44	0.056	0.014	36.0	32.0	34.0	26.3	24.6		35.2	31.5	34.1	31.0	28.5	
11	Askov Sandy field	sandy soil	none	sl. acid	0	0.069	1.80	0.049	0.013	36.4	36.0	33.7	33.5	32.8	32.7	37.3	37.2	33.5	33.3	34.1	30.4
12	Faroe Islands	brownish sandy soil, rich in humus	none	sl. acid	0	0.778	0.62	0.136		8.2	1.0					11.3	1.6				
13	Gårding S. H.	v. l., gray sandy soil	none	sl. acid	0	0.021	0.24	0.220	0.008							38.3	36.8	32.4	25.1	12.6	
14	Haverslev	sandy soil	none	sl. acid	0	0.066	0.54	0.220	0.016	40.7	39.0	34.6	28.2	15.6		39.5	38.5	35.4	27.6	16.6	
15	S. P. F.	l., dark gray sandy soil	none	sl. acid	0	0.079	1.28	0.353	0.028							36.4	21.6	9.2			
16	Hornum	sandy soil	none	sl. acid	0	0.078	1.79	0.101	0.004	40.4	38.9	38.4	32.2	25.8		40.5	38.3	36.4	25.5	11.1	
17	Aalsø	sandy soil	none	neut. sl. acid	0	0.094	1.18			39.5	39.0	35.3	16.0	1.9		35.1	25.4	3.4			

68	Grindsted	l., dark gray coarsely grained sandy soil	none	neut. sl. acid	0	0.037	0.66	0.101	0.0	36.8	30.0	22.2	17.6	10.1	5.6	28.2	30.6	20.7	16.1	19.3	17.7
69*	Fedgaard	dark gray, rich in humus	none	neut. sl. acid	0	0.017	2.26	0.166		20.1	5.4	1.0				21.7	3.9	1.0			
70	Askov III	l.	none	neut. sl. acid	0	0.082	2.14	0.115	0.014	37.1	36.1	36.8	34.6	32.1		35.8	34.7	33.1	29.1	23.5	
71	Skrold, Gandrup	l. gray soil	none	neut. sl. acid	0	0.064	0.64	0.112	0.023	39.4	38.4	31.1	7.9			39.5	38.4	27.9	4.9		
72	Gaaser, Gandrup	l. gray	none	neut. acid	0	0.084	2.22	0.131	0.021	38.1	35.9	13.7	3.1			36.9	34.4	10.3	3.2		
73	Skovsgaard, Højby	v. f. grained, r. rich in humus	none	neut.	0	0.068	1.72	0.408	0.029	40.7	38.3	32.4	22.9	4.4		39.5	38.9	32.6	21.9	4.1	
74	Bregentved	l.	none	neut.	0	0.075	1.09	0.239	0.021	38.3	35.1	29.1	5.6	1.8		38.0	31.1	15.2	3.2		
75	Thustrup, Skjæring	f. grained	none	neut.	0	0.123	2.72	0.266	0.027	38.4	37.6	28.4	4.4	4.2		36.8	22.7	4.1			
76	Enslev, Gjerlev	v. f. grained	none	neut.	0-1	0.094	1.84	0.292	0.018	39.1	25.8	1.6				29.4	4.3				
77	Stadsgaard	l. gray	none	neut.	1	0.056	0.74	0.251	0.010	38.1	38.2	37.9	36.4	31.0		38.2	37.6	35.9	32.6	27.1	
78	Nejsgaard	none	none	neut.	3	0.070	1.00	0.120	0.026	39.1	3.5					5.8	3.8				
79	Askov Sand- mark, G. 2	v. sl.	v. sl.	neut.	3	0.082	1.16	0.202	0.008	36.7	39.6	3.0				2.9					
80	Elkenør	v. l. r. coarsely grained, sl. decomposed	none	neut.	4	0.048	1.80	0.223	0.021	37.5	32.9	21.1	11.4			35.7	26.8	17.1	7.2		
81	Toftsgaard, Gaaser	l. gray	v. sl.	neut.	4	0.098	4.34	0.308	0.042	38.5	3.8					2.3					
82	Thorsø, Grønaa	l.	none	neut.	4	0.072	2.94	0.188	0.018	38.9	35.5	33.4	17.3	3.9		37.1	36.5	32.0	17.0	8.0	
83	Hagedstedt, Maarsø	v. f., v. poor in humus	none	neut.	4	0.070	1.04	0.236	0.022	38.6	36.7	34.3	27.4	8.4		37.6	35.0	24.0	7.3	4.2	
84	Skindbjerg II	l.	v. sl.	neut. sl. alk.	3	0.085	3.22	0.231	0.022	13.2	4.0					4.8	3.5				

TABLE 2—Continued

Number	Soil	GENERAL CONDITION OF THE SOIL	ACID REPERFERENCE	REACTION	DEVELOPMENT OF AZOTOACTIVITY†	PHOSPHORIC ACID CONTENT		AMMONIUM CHLORIDE SOLUBLE	CONTENT OF ORGANIC MATTER AFTER VARIOUS DECOMPOSITION PERIODS ‡											
						P <sub>2</sub> O <sub>5</sub> soluble in m.-n. ratio acid	P <sub>2</sub> O <sub>5</sub> per filter of (C) <sub>2</sub> H <sub>5</sub> O saturated water		Uninoculated						Inoculated					
									5 days	10 days	15 days	20 days	25 days	30 days	5 days	10 days	15 days	20 days	25 days	30 days
Sandy soils—Continued																				
85	Skindbjerg I		none	reut. sl.	4	per cent 0.064	mgm. 0.92	per cent 0.219	cc. # 0.018	cc. 34.5	cc. 11.0	cc. 3.2	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
86	Enselevgaard, Grenaa	r. rich in humus	str.	sl. alk.	4	0.095	4.48	0.924	0.025	17.9	3.5					7.1	3.7			
87	Fladsaagaard, Næstved	l., r. coarse	sl.	sl. alk.	4	0.150	2.42	0.729	0.023	19.4	3.5					6.8				
88	Aastrup	rich in humus	r. str.	alk.	4	0.095	2.34	0.646	0.029	4.9	2.6					3.1				
89	Petersdal, Kastrup	coarse, rich in humus	str.	alk.	4	0.143	12.2	0.521	0.029	33.3	13.9	5.2				4.9	2.2			
90	Gjerrild	r. rich in humus	str.	alk.	4	0.114	8.46	0.500	0.020	3.4						21.4	3.1			
91	Teglærsgd., Jyderup	l., poor in humus	r. str.	alk.	4	0.081	1.61	0.332	0.013	28.8	3.2					2.9				
92	Mørkøv	v. l., coarse	r. str.	alk.	4	0.075	1.75	0.435	0.012	9.0	5.7					6.9				
93	Trustrup brick works		v. str.	str. alk.	4	0.083	2.54	0.567	0.018	2.9						3.0				
94	Hulmosegd., Nyraad	v. f. grained, poor in humus	str.	str. alk.	4	0.054	1.55	0.406	0.016	36.0	30.6	22.3	5.3			2.9				
95	Thisted field	r. rich in humus	r. str.	str. alk.	4	0.110	2.74	0.693	0.025	6.1						30.9	12.0	3.1		

exists between the reaction of the soil, particularly its content of buffers, measured by its azotobacter development in the azotobacter test, and its power of decomposition. The *non-lime-requiring soils* with azotobacter development possess, as a rule, a far greater power to decompose mannite than the lime-requiring soils without azotobacter development. The author found this in his earlier investigations (1) on the decomposition of peptone and cellulose. Many exceptions, however, are found which show that the relation depends to a large extent on some factor other than lime-requirement. If we confine ourselves solely to the strongly acid soils, we observe that these, with but a single exception (table 2), possess a comparatively slight power to decompose mannite.

Earlier investigations of the author (1) on the influence of the condition of the soil on the decomposition of various organic substances showed that the content of phosphoric acid in a form available for the microorganisms in question was particularly effective in determining the speed of decomposition. The influence of this factor in this investigation was noted by determining, in the case of most of the soil samples, the content of phosphoric acid present in combinations soluble in boiling 20 per cent muriatic acid and in carbonic acid.<sup>4</sup> Determinations were likewise made of the lime- and magnesia-content of the soil by a method previously described by the author (2).

No connection can be shown between the content of phosphoric acid in muriatic-acid-soluble combinations and the speed of mannite decomposition. However, the results (disregarding the group of non-basic soils) in which the conditions for mannite decomposition, as mentioned above, are poor, indicate definitely that those soils showing the greatest saturation-concentration of  $P_2O_5$  in carbonic-acid-saturated water possessed as a rule the greatest power to break down mannite.

Non-lime requiring inoculated soils which had given off more than 1.2 mgm.  $P_2O_5$  (per liter of  $CO_2$ -saturated water), decomposed practically all the mannite applied within 10 days. On the other hand, soils which had not given off more than 0.5 mgm. of  $P_2O_5$  possessed a very small power of mannite decomposition. No absolute relation can be shown between the saturation-concentration of  $P_2O_5$  and the speed of decomposition within the limits of these values. In the case of soils with a pronounced alkaline reaction, the above-named values seem to lie within much narrower limits, for in every case in which the saturation-concentration exceeds 0.5 mgm.  $P_2O_5$  per liter, a rapid mannite decomposition has taken place in the inoculated cultures.

A particularly striking example of the influence of phosphoric acid on the speed of mannite decomposition may be seen by considering the results obtained in using soil 43, described on page 350. It is the only one of the strongly alkaline-

<sup>4</sup>The method used by Mitscherlich (5) in determining the saturation-concentration of  $P_2O_5$  in carbonic-acid-saturated water as the expression of the solubility of the phosphoric acid in soil, is used with small modifications. The process of decomposition takes place at a temperature of 30°C.

reacting soils which caused a very slow mannite decomposition. The saturation-concentration of  $P_2O_5$  in carbonic-acid-extract was extremely small, (0.26 mgm. per liter).<sup>5</sup> We observe, too, that several of the non-lime-requiring soils, even though they had a small saturation-concentration of  $P_2O_5$  in carbonic-acid-extract, caused rapid mannite decomposition.

It was observed that in some of the soils 3-6 days after the decomposition experiment had begun, slimy azotobacter coatings appeared. A closer inspection, as a rule, showed that the entire soil portion was filled with these coatings, often rendering it sticky. This would seem to indicate a rather large content of easily soluble, phosphoric-acid combinations (table 3).

It is possible that some other soils tested showed the azotobacter coating described, but they were not examined systematically. In the investigations

TABLE 3

SOIL NUMBER	LITMUS REACTION	CaCO <sub>3</sub> SOLUBLE IN AMMONIUM CHLORIDE	P <sub>2</sub> O <sub>5</sub> IN COMBINATIONS SOLUBLE IN MURIATIC ACID	SATURATION-CONCENTRATION OF P <sub>2</sub> O <sub>5</sub> IN CARBONIC ACID EXTRACT	TIME FOR COMPLETE DECOMPOSITION IN INOCULATED CULTURES
		per cent		mgm. per liter	days
18 (table 3)	Neutral	0.21	0.144	8.70	5
29 (table 3)	Neutral	0.27	0.118	4.86	5
31 (table 3)	Neutral	0.27	0.087	1.44	5
33 (table 3)	Neutral	0.39	0.114	2.38	5
44 (table 3)	Alkaline	0.41	0.177	10.00	5
9 (table 5)	Alkaline	0.58	0.137	1.10	5
10 (table 5)	Alkaline	0.52	0.123	3.34	5
a*	Alkaline	0.37	0.062	2.16	10
b*	Alkaline	0.26	0.112	6.52	5

\* These soils belong to another series of investigations and are therefore not quoted in any of the tables of the present paper.

<sup>5</sup> A decomposition experiment with the same soil samples was made about 12 months later. During that time the soil had lain in an air-dry, pulverized condition in a glass jar placed in the store-room of the laboratory. The decomposition experiment was made with "inoculated" cultures. It was observed that mannite decomposition took place much more quickly than the first time. The values expressing mannite decomposition may be compared as follows:

Year of experiment	Mgm. P <sub>2</sub> O <sub>5</sub> per liter CO <sub>2</sub> saturated water	Organic-matter content after:					
		5 days	10 days	15 days	20 days	25 days	30 days
1919	0.26	40.4	38.1	36.8	35.9	30.3	25.3
1920	0.85	36.0	22.9	6.2			

The solubility of phosphoric acid in the soil was considerably greater in the soil sample that has been stored than in the comparatively fresh one and in relation to the experiments already described it seems reasonable to suppose that this storage was the cause of the more rapid decomposition of mannite. Rahn (6) has shown that air-drying increased this power considerably, and in view of the very important influence, which phosphoric acid exerts on the various biological changes in matter in the soil, it may be considered probable that this increase has been caused by the effect of air-drying on the solubility of the phosphoric acid in the soil. Various investigations (4) indicate also that air-drying of soil increases the solubility of various substances in it.

described later (table 4), with direct addition of a large quantity of phosphates, the azotobacter coating appeared very frequently in the "phosphate" tumblers, but never in those tumblers to which no phosphates were added. Azotobacter coating in soil under conditions present in ordinary decomposition experiments, may, then, plainly enough be considered a qualitative, biological, phosphoric-acid reaction. It is probable, however, that a visible azotobacter coating may require a larger content of these combinations than is necessary to satisfy the need of ordinary crops.

In the case of soils with an acid reaction, and particularly in acid loamy soils, no connection can be found between the variations in the saturation-concentration of  $P_2O_5$  in carbonic-acid extracts and the speed of mannite-decomposition. These soils, with a few exceptions among sandy soils, always broke down mannite slowly.

The exceptions were the two sandy soils rich in humus, no. 62 (Faroe Islands) and no. 69 (Fedgaard), which have just as much claim to be considered humus soils as mineral soils. Humus soils seem to show a very different relation to mannite decomposition than mineral soils. They often contain a comparatively large amount of phosphoric-acid combinations soluble in carbonic acid. There will probably be occasion to return later to this problem which must be made the subject of special investigations.

The relation between the soil's content of lime soluble in ammonium chloride and the course of mannite-decomposition does in the main correspond to that described for the reaction of the soil. That a considerable content of the nutritive substance calcium cannot neutralize the restraining effect which the lack of basic substances in the soil exerts on the decomposition of mannite, is often proved in the results. This is particularly apparent in the investigations of soil 73.

It seems impossible to show any relation between the soil's content of magnesia soluble in ammonium chloride (which indeed varies within much narrower limits than the content of lime soluble in ammonium chloride) and its power to break down mannite. This may possibly be explained by the fact that no relation exists between the content of magnesia and soil reaction (cf. table 2.)

As table 2 shows, mannite decomposition as a rule took place somewhat more quickly in the "inoculated" than in the "uninoculated" cultures. In a few of the soils (15, 32, 40 and 67), the difference was quite apparent. This expresses the fact, perhaps, that the change in the microbiological conditions of the soil did not keep pace with the possible changes which took place in the chemical condition. In most cases the difference disappeared at the end of five or ten days, which shows that the majority of the cultivated mineral soils contain a sufficient number of mannite-decomposing organisms to enable it to attain an approximately maximum decomposition-speed, and that the difference in power of mineral soils to break down mannite is primarily due to differences in their chemical composition.

TABLE 4  
The influence of various substances on manure decomposition in the soil (inoculated cultures)

KIND OF SOIL AND NUMBER	ACID EFFERVESCENCE	LITMUS REACTION	DEVELOPMENT OF AUTO-BACTER	TREATMENT*	CONTENT OF ORGANIC MATTER AFTER VARIOUS DECOMPOSITION PERIODS†							
					5 days	10 days	15 days	20 days	25 days	30 days		
					cc.†	cc.	cc.	cc.	cc.	cc.	cc.	cc.
Loamy soils												
<i>Forles</i> (1917) (No. 26, Table 2) r. h.	none†	neut.†	4	none.....	32.3	26.9	24.4	20.8	15.1	cc.		
				CaCO <sub>3</sub> .....	29.9	6.0	1.1					
				CaCO <sub>3</sub> + CaHPO <sub>4</sub> .....	27.4	1.0						
				CaCO <sub>3</sub> + KCl.....	33.2	5.8						
				CaCO <sub>3</sub> + KCl + CaHPO <sub>4</sub> .....	32.5	1.1						
				CaCO <sub>3</sub> + K <sub>2</sub> HPO <sub>4</sub> .....	29.4	0.9						
<i>Støpdy</i> (No. 21, Table 2) h.	none	neut.	3	K <sub>2</sub> HPO <sub>4</sub> .....	28.5		8.1	3.8				
				none.....	27.1	28.6	27.1	14.1	2.4			
				CaCO <sub>3</sub> .....	18.1	0.7						
				CaCO <sub>3</sub> + CaHPO <sub>4</sub> .....	1.1							
				CaCO <sub>3</sub> + KCl.....	13.0	0.5						
				CaCO <sub>3</sub> + KCl + CaHPO <sub>4</sub> .....	1.1							
<i>Åsboe</i> (No. 8, Table 2)	none	sl. acid	0	CaCO <sub>3</sub> + K <sub>2</sub> HPO <sub>4</sub> .....	0.9							
				K <sub>2</sub> HPO <sub>4</sub> .....	21.2	17.6	19.7	6.9	3.7			
				none.....	34.5	29.3	27.5	27.2	24.8	15.6		
				CaCO <sub>3</sub> .....	33.9	30.3	1.6					
				CaCO <sub>3</sub> + CaHPO <sub>4</sub> .....	28.8	1.2						
				CaCO <sub>3</sub> + K <sub>2</sub> HPO <sub>4</sub> .....	20.2	1.6						
				CaHPO <sub>4</sub> .....	34.8	31.6	24.4	16.6	0.9			

<i>Askov (B 3)</i> (No. 43, Table 2)	v. sl.	alk.	4	K <sub>2</sub> HPO <sub>4</sub> .....	33.3	30.8	26.1	18.3	8.2	1.4
				KH <sub>2</sub> PO <sub>4</sub> .....	35.5	†	30.9	20.4	10.8	1.0
				none.....						
				CaCO <sub>3</sub> .....	39.5	36.1	27.3	15.6	7.5	
				CaCO <sub>3</sub> + CaHPO <sub>4</sub> .....	39.1	36.8	24.9	11.9	3.1	
				CaCO <sub>3</sub> + KCl.....	4.1					
				CaCO <sub>3</sub> + CaHPO <sub>4</sub> + KCl.....	39.1	37.4	29.3	18.9	10.0	
				CaCO <sub>3</sub> + CaHPO <sub>4</sub> + KCl + (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	3.1					
				CaCO <sub>3</sub> + CaHPO <sub>4</sub> .....	1.3					
				CaHPO <sub>4</sub> .....	2.5					
<i>Slangerup (1917)</i> (No. 37, Table 2)	v. sl.	sl. alk.	4	K <sub>2</sub> HPO <sub>4</sub> .....	2.3					
				CaCO <sub>3</sub> + K <sub>2</sub> HPO <sub>4</sub> .....	2.0					
				CaCO <sub>3</sub> + KH <sub>2</sub> PO <sub>4</sub> .....	4.1					
				none.....	32.2	26.0	17.0	0.1		
				CaCO <sub>3</sub> .....	29.4	24.0	16.0	0.0		
				CaCO <sub>3</sub> + CaHPO <sub>4</sub> .....	25.1	25.4	14.6	0.1		
				CaCO <sub>3</sub> + CaHPO <sub>4</sub> + KCl.....	29.8	26.6	25.0	6.3	0.2	
				CaCO <sub>3</sub> + K <sub>2</sub> HPO <sub>4</sub> .....	31.7	21.7	5.3	0.1		
				K <sub>2</sub> HPO <sub>4</sub> .....	31.4	21.8	5.0	0.2		
				<i>Slangerup (1919)</i>	v. sl.	sl. alk.	4	none.....	34.0	20.4
CaCO <sub>3</sub> + CaHPO <sub>4</sub> + K <sub>2</sub> SO <sub>4</sub> .....	4.4									
CaCO <sub>3</sub> + CaHPO <sub>4</sub> + K <sub>2</sub> SO <sub>4</sub> + (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	3.3									
CaCO <sub>3</sub> .....	35.6	20.5	0.3							
<i>Lyngby I</i> 1., r. poor in humus	none	acid	0	CaCO <sub>3</sub> .....	31.4	1.0				
				CaCO <sub>3</sub> + CaHPO <sub>4</sub> .....	31.8	1.0				

\* Of the various substances the following quantities are used per tumbler (75 gm. soil) 0.75 gm. CaCO<sub>3</sub>; 0.30 gm. CaHPO<sub>4</sub>; 0.15 gm. K<sub>2</sub>HPO<sub>4</sub>; 0.113 gm. KH<sub>2</sub>PO<sub>4</sub>; and 0.075 gm. KCl.

† See footnote to table 1. These data are also averages.

‡ As in this determination a serious error has probably been made; the average is not calculated.

§ Abbreviations used are: alk. = alkaline, h. = heavy, l. = light, neut. = neutral, r. = rather, sl. = slight or slightly, v. = very.



TABLE 4—Continued

KIND OF SOIL AND NUMBER	ACID EFFERVES- CENCE	LITMUS REACTION	DEVELOP- MENT OF AZOTO- BACTER	TREATMENT*	CONTENT OF ORGANIC MATTER AFTER VARIOUS DECOMPOSITION PERIODS†						
					5 days	10 days	15 days	20 days	25 days	30 days	
Loamy soils—Continued											
<i>Lyngby P.</i> poor in humus	none	acid	0	none.....	cc.†	cc.	cc.	cc.	cc.	cc.	cc.
				CaCO <sub>3</sub> .....	38.5	35.2	22.4	6.6	3.3		
				CaCO <sub>3</sub> + CaHPO <sub>4</sub> .....	37.4	30.7	9.2	1.5			
<i>Aakirkeby</i> h.	none	acid	0	none.....	35.8	5.1	2.1				
				CaCO <sub>3</sub> .....	33.8	20.3	7.6				
				CaCO <sub>3</sub> + CaHPO <sub>4</sub> .....	24.6	0.9					
<i>Kildebrynde</i> h.	none	sl. acid	0	none.....	14.6	0.8					
				CaCO <sub>3</sub> .....	38.7	32.4	23.4	16.5	9.5		
				CaCO <sub>3</sub> + CaHPO <sub>4</sub> .....	5.1						
Sandy soils											
<i>Landsgaard II</i> (No. 60, Table 2) l.	none	sl. acid	0	none.....	33.9	34.4	33.1	26.8	19.6	13.4	
				CaCO <sub>3</sub> .....	(31.4)	34.1	30.3	21.0	2.6		
				CaCO <sub>3</sub> + CaHPO <sub>4</sub> .....	27.7	25.1	4.7	1.7			
				CaCO <sub>3</sub> + K <sub>2</sub> HPO <sub>4</sub> .....	36.1	28.4	24.3	5.6	1.9		
				CaHPO <sub>4</sub> .....	(29.6)	34.5	31.7	20.3	17.7	12.1	
				K <sub>2</sub> HPO <sub>4</sub> .....	35.6	31.5	29.7	30.9	21.4	18.7	
				KH <sub>2</sub> PO <sub>4</sub> .....	34.9	31.2	28.2	21.6	11.3	6.0	
<i>Stadsgaard</i> (No. 77, Table 2)..... l. gray	none	neut.	1	none.....	38.6	37.6	33.5	26.3	17.7		
				CaCO <sub>3</sub> + CaHPO <sub>4</sub> .....	37.5	36.5	28.5	18.8	10.4		



Table 4 gives the influence of *chemical factors* on the decomposition of mannite in soils. The principles involved have been described (1). Soil samples which previous investigations had shown to possess a comparatively slight power to break down mannite were used. The method of procedure may be seen in the table. Only "inoculated" cultures were used.<sup>6</sup>

The results of these investigations show that *the soil's content of basic lime and of phosphoric acid combinations determine the speed at which mannite decomposition takes place*. As the author has already shown, 1914 (1) in the report of investigations of the decomposition of cellulose, carbonate of lime may further decomposition in four ways; (1) by acting directly as lime nutrition on the microbes in question; (2) by changing the reaction; (3) by giving acid saturation (buffer effect), and (4) by transforming the plant nutritive substances in the soil that are soluble only with difficulty, into a form readily assimilable by the micro-organisms in question. While the investigations of the requirements necessary for cellulose decomposition in mineral soils showed that the first effect was of but little value for this process which transpired just as quickly when di-basic potassium phosphate ( $K_2HPO_4$ ) was used as when this phosphate was used in connection with carbonate of lime, the results of the present investigations revealed the fact that the calcium-ion itself is of great importance in the work done by the mannite decomposing microbes. The use of di-basic potassium phosphate, in spite of its buffer effect and power to change the reaction toward the alkaline, has repeatedly resulted in a far slower mannite-decomposition than the use of that salt or of calcium phosphate in combination with carbonate of lime. This relation appears very plainly in decomposition experiments using soil with an acid reaction from Lundgaard. Here the use of potassium phosphate alone has had no effect whatsoever on mannite-decomposition. In loamy soil from Askov with acid reaction and the sandy soil from Studsgaard the effect of  $K_2HPO_4$  used alone is small in comparison with the effect obtained by using this salt in combination with  $CaCO_3$ . Di-basic potassium phosphate, moreover, seemed under certain conditions to exert a restraining influence on mannite-decomposition.

That the power of carbonate of lime to change reaction and cause acid-saturation can, under given conditions, have an important effect on the speed of mannite-decomposition, may be seen very plainly in the investigations of the two lime-requiring soils (acid reaction and no azotobacter development) from Askov and Lundgaard. The addition of carbonate of lime to di-basic phosphate of lime ( $CaHPO_4$ ) to these soils greatly stimulated mannite decomposition. Of the soil from Lundgaard, the portion mixed with calcium phosphate alone

<sup>6</sup> Note must be made of the fact that several of these investigations, those, for instance, with soil from Lundgaard, were made before the methods of analysis was sufficiently developed. This may explain why the values found for the content of organic matter at the close of a period can be greater than in the preceding period, a circumstance not encountered after the improved analysis method was brought into use. However, the results obtained threw sufficient light on the matter in question to warrant their being included here.

did not perceptibly break down mannite more quickly than the soil portion to which nothing had been added. Only when both carbonate of lime and phosphate of lime were added was the process hastened to any appreciable degree.

Whether the power of carbonate of lime to stimulate mannite-decomposition depends on its power to render more active the difficultly soluble phosphoric-acid-combinations in the soil, cannot be answered positively on the basis of the results of these experiments (1, p. 132-34 German translation). It seems probable, however, that the very stimulating effect which carbonate of lime used alone had on mannite-decomposition in soils poor in phosphates soluble in carbonic acid, depends largely on that power.

It is possible also that the power which lime possesses of rendering difficultly soluble phosphoric-acid-combinations more soluble is responsible for the often observed slight stimulation of mannite-decomposition after the addition of easily soluble phosphates as a supplement to lime. On the whole, the results show plainly enough the great influence exerted by the presence of easily soluble phosphoric-acid-combinations on the speed of mannite-decomposition. On the other hand, the addition of potassium in the form of potassium chloride did not stimulate mannite-decomposition. Furthermore, since  $K_2HPO_4$  in no instance caused mannite to break down more rapidly than  $CaHPO_4$  alone or in combination with  $CaCO_3$ , we may conclude that the potassium ion seems to have no effect or only a very slight effect on that process. In 1914 in investigations of the conditions necessary for cellulose and peptone decomposition in the soil, the author (1) obtained similar results.

While the investigations referred to seem to prove that the speed of mannite-decomposition depends in the main on the reaction of the soil and its buffer content, as well as on its content of easily soluble phosphoric-acid-combinations, the present material also indicates that there are other important factors. We find, for instance, that the addition of  $CaCO_3 + CaHPO_4$  does not stimulate mannite-decomposition equally in all soils. In the soil from Strøby, for instance, mannite-decomposition was complete at the end of 5 days while in soil from Lungaard a considerable portion of undecomposed organic matter remained in the tumbler after 10 days. In the soil from Slangerup (1917), which was drawn from unfertilized plots in an experiment with fertilizers,  $CaCO_3$  alone or in combination with  $CaHPO_4$  had no stimulating effect on decomposition. When  $K_2HPO_4$  was added there was slight stimulation. That the content of potassium was not the important factor here may be seen from the fact that KCl had no effect when added to  $CaCO_3 + CaHPO_4$ . In a decomposition experiment made two years later with a new soil sample from the same plots, table 4, when carbonate of lime and calcium phosphate were added to the soil mannite decomposition was complete after 5 days.

There is reason, then, to expect that a determination of the power of soil to break down mannite may to some extent express its content of easily soluble phosphoric-acid-combinations and perhaps, too, of those readily assimilable by plants. As preliminary study of the question which is great importance

for practical soil investigations, an examination was made of certain soils whose need of phosphoric acid had been determined by field experiments carried out through many years. Unfortunately there are only a few experiments here in Denmark which can supply material for these investigations. This is much to be regretted both in the present work and in further studies which will aim at explaining the part played by the various substances in the soil.

The material used is from the following experiments.

Soil samples 1 and 2 from the permanent experiments with stable manure and artificial fertilizers on the "loamy field" at Askov Experiment Station (Field B 3) were a light loam rather dark in color. No. 1 was drawn from the plots which have been unfertilized since 1893. No. 2 was from plots fertilized only with superphosphates. From 1893 to 1907 85 kgm. of 18-per cent superphosphate was applied per Td<sup>7</sup> land per year. The following years 95 kgm. was applied annually. The sample was drawn in the autumn of 1919. A comparison between the plant production, (even though this was but scanty in both instances) on the phosphate fertilized and the unfertilized plots showed plainly that the soil in the latter plots was greatly in need of phosphoric acid.

Soil samples 3 and 4 from corresponding experiments begun at the same time on the "sandy field" at Askov Experiment Station (Field G-2) were light sandy soil long cultivated. Sample 3 was drawn from the unfertilized and sample 4 from plots fertilized only with superphosphates. The samples were drawn in the autumn 1918. Neither in this experiment nor in other experiments from the "sandy field" of Askov Experiment Station did the addition of superphosphate produce any effect (3).

Soil samples 5, 6, 7, and 8 from the "sandy field" of Askov Experiment Station were drawn during the winter of 1920 from an experiment with artificial fertilizers on alfalfa begun in its final form in 1899. As in the preceding experiment, this was a light sandy soil. Sample 5 was from an unfertilized plot, sample 6 from a plot treated with 150 kgm. Thomas phosphates per Td. land annually, sample 7 from a plot treated with 200 kgm. kainit per Td. land annually, and sample 8 from a plot treated with 200 kgm. kainit and 150 kgm. Thomas phosphates per Td. land annually. All the samples showed acid reaction, so the decomposition experiments were made both with and without the admixture of carbonate of lime (1 per cent of the weight of the soil). While the plant growth showed a considerable reaction from the addition of potassium, neither sample 6 nor sample 8 showed any reaction from the addition of Thomas phosphates (3).

Soil samples 9-10 were drawn from a starvation experiment on the demonstration field of the Royal Agricultural College in Copenhagen (9). The soil is a heavy loam, rich in humus. Sample 9 was drawn from plots which since 1898 have been fertilized annually with 30 kgm. nitrogen, (in the form of Chili saltpeter) and 30 kgm. potassium (in the form of potassium sulfate) per Td. land, and thus starved for phosphoric acid. Sample 10 was drawn from plots which in addition to the amount of nitrogen and potassium mentioned above, had been treated annually with 18-per cent superphosphates, containing 20 kgm. of  $P_2O_5$ . The samples were drawn in March, 1919. No effect from the addition of phosphates has been observed hitherto.

Samples 11 and 12 were drawn October, 1917, from a starvation experiment at Tystofte Experiment Station. The soil was a heavy loam, poor in humus. Sample 11 was drawn from plots which, since 1913, have been treated annually with an average of 175 kgm. Chili saltpeter and 138 kgm. 37-per cent potassium fertilizer per hectare and thus starved for phosphoric acid. Sample 12 was drawn from plots which, in addition to the fertilizers named above, had been treated annually with 200 kgm. 18-per cent superphosphates per hectare. There was a distinct effect of phosphoric acid starvation in the plots to which no phosphoric acid fertilizer was added, observable in the plant growth.

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<sup>7</sup> A Td. (Tønde) land = 0.55 hectare.

Soil samples 13, 14, 15 and 16 were drawn in November 1920 from an experiment begun in the spring of 1916 at Smakkebakgaard near Lundby, Seeland. The soil is a rather heavy loam, which is very productive. The samples were taken from plots receiving the following treatments: 13, Chili saltpeter; 14, Chili saltpeter and superphosphates; 15, Chili saltpeter and potassium fertilizer; 16, Chili saltpeter, potassium fertilizer and superphosphate. All the crops, though particularly barley, on the plots represented by samples 14 and 16 showed considerable response to the phosphoric acid fertilizer.

Soil samples 17 and 18 were drawn in April, 1921, from an experiment begun in 1916 at Kalby, Seeland, on a very productive loamy soil. Plot 17 had been unfertilized while plot 18 had received treatment of superphosphate. No effect from the addition of superphosphates had been observed.

Soil sample 19: In an experiment field at Oelstykke, Seeland, on sandy soil, 300 kgm. of superphosphate per hectare as a supplement to 200 kgm. Norwegian saltpeter and 150 kgm. of potassium fertilizer increased the yield of swedes<sup>a</sup> by 15 per cent. In 1920, sample 19 from soil adjacent to the experiment was drawn to be used at the Royal Agricultural College in a pot experiment with barley. It was found that easily soluble phosphates (54 kgm.  $P_2O_5$  per hectare) increased the crop by 11-19 per cent. At the close of the experiment, samples were taken from the pots which had received nitrogen-potassium fertilizer. Since the soil showed an acid reaction to litmus, lime was added to half of the samples (see further table 4).

Sample 20 was drawn in 1921 from the unfertilized plots of an experiment at Blaaehøj, Grindsted (Jutland) on a dark gray, sandy soil rather rich in humus, but unproductive. The experiment was begun in the spring of 1920, with swedes as the experimental crop. Plot treatments were: (a) unfertilized, (b) 200 kgm. Norwegian saltpeter, (c) 200 kgm. Norwegian saltpeter and 300 kgm. of 18-per cent superphosphates. We have results from the one year only, but the effect from superphosphates was so enormous that no further proof of the great phosphoric acid requirement of the soil was necessary. The addition of superphosphate more than quadrupled the yield. On account of the acid reaction, this sample was set aside both with and without lime. (Cf. table 4.)

The results of the investigations of these soils are given in table 5.

After the differences in the microorganism flora which cause mannite decomposition are removed by inoculation, the inoculated cultures express the influence which the differences in the *chemical* condition of the soil exert on mannite decomposition. *In those experiments in which the addition of phosphoric acid has considerably increased the yield, all the soil samples from plots not fertilized with phosphoric acid possess only a very slight power of mannite decomposition.* Out of this group, however, samples from plots in the three permanent experiments (Askov "loamy field," Tystofte and Lundby), which for several years had been treated with superphosphates, possess a very great power of mannite-decomposition. In the soil from Askov mannite-decomposition was well advanced after 5 days, and in all three soils it was complete after 10 days. In the two decidedly lime-requiring soils within this group (from Oelstykke and Grindsted) mannite decomposed very slowly and the limed samples were only slightly ahead of the unlimed samples.

These results agree closely with the results from three of the four fertilization experiments in which no effect was observed from the addition of superphosphates. These three are the experiment with stable manure and artificial fertilizers on

<sup>a</sup> A swede is *Brassica Napus L. rapifera*.

TABLE 5  
*Mannite decomposition in soils from field experiments with phosphoric acid fertilizer*

SAMPLE NUMBER	ACID DEVELOP- MENT	LITMUS REACTION	DEVELOP- MENT OF ACIDOTY- PICAL BACTERIA	PHOSPHORIC ACID CONTENT			EFFECT OF PHOSPHORIC ACID FERTILIZER IN FIELD	CONTENT OF ORGANIC MATTER AFTER VARIOUS DECOMPOSITION PERIODS†											
				P <sub>2</sub> O <sub>5</sub> soluble in 100 ml. of 10% acid water	P <sub>2</sub> O <sub>5</sub> per liter of acid water	mgm.		Uninoculated culture					Inoculated culture						
								per cent	5 days	10 days	15 days	20 days	25 days	30 days	5 days	10 days	15 days	20 days	25 days
1	v. weak	alk. ¶	4	0.046	0.26		str. ¶	39.8	37.6	36.7	37.5	35.7	35.4	40.4	38.1	36.8	35.9	30.3	25.3
2	weak	alk.	4	0.058	2.08			38.7	7.7	3.1				19.4	2.4				
3	weak	neut. sl. alk.	4	0.078	1.56		none	37.8	—	4.7	3.0			2.9					
4	weak	neut. sl. alk.	4	0.092	2.42			37.9	40.2	—	—	—	0.5	2.7					
5	none	neut. sl. acid	0	0.075	1.96		none							37.6	39.6	35.1	24.6	12.0	
6	none	neut.	0	0.088	4.20									33.2	20.6	5.1	30.3	22.7	
7	none	neut. sl. acid	0	0.074	1.0		none							37.5	38.4	35.4	29.0	20.0	
8	none	neut. sl. acid	0	0.080	2.50									37.2	38.1	33.7	29.0	20.0	
5a*														33.8	14.8	2.8			
6a														21.2	1.9				
7a														33.7	17.0	5.5			
8a														33.1	16.2	2.3			
9	weak	alk.	4	0.137	1.10		none	32.5	5.6					3.6					





the Askov "sandy field," that on the field of the Royal Agricultural College and that at Kalby. Soil from plots to which no phosphoric acid was added possessed, in each case, great power of mannite-decomposition. In every case this was complete after 5-10 days. In these experiments there can be no question of a difference in speed of mannite-decomposition in soil samples from the non-phosphoric acid fertilized and the phosphoric acid fertilized plots. In the experiment on Askov "Sandy field" mannite decomposition was very slow both in plots fertilized with phosphoric acid and in plots not fertilized with phosphoric acid. This is, undoubtedly, due to the decidedly acid reaction of the soil samples in question. When the soil samples were treated with carbonate of lime, mannite-decomposition was in every instance, comparatively rapid. Table 5 shows that soil from plots which received potassium-fertilizer only possessed, after the addition of lime, just as great a power of mannite-decomposition as soil in plots receiving both potassium-fertilizer and Thomas phosphates. On the other hand, strangely enough, soil fertilized with Thomas phosphates alone showed, in both series of experiments a greater power of mannite-decomposition than any of the other soil samples drawn. The cause of this may possibly lie in the fact that this soil contained a very large content of phosphoric acid in combinations soluble in carbonic acid. The saturation-concentration of phosphoric acid in the carbonic-acid extract varied greatly in this experiment; i. e., from 1.0 on the kainit plots, to 4.20 on the Thomas-phosphate plots. But even in soil from the kainit plots soluble phosphoric-acid combinations were found in quantities sufficient for both plant nourishment and rapid mannite-decomposition.

In the other experiments, too, there was a very considerable difference in phosphoric-acid content in carbonic-acid-extracts of soils from the non-phosphoric acid and phosphoric acid fertilized plots. This difference was relatively greatest in the experiment on Askov "loamy field." Furthermore the *saturation-concentration of  $P_2O_5$  in carbonic-acid-extracts, both absolutely and relatively, was very small in all the five soils with distinct phosphoric-acid-requirement.*

The sensitiveness of the speed of mannite-decomposition to the lack of phosphoric acid in the soil appeared very plainly in the investigations of soil samples from experiments at *Tystofte* and *Lundby* (conducted for only a very few years). The amount of phosphoric acid which had been added to the superphosphate-fertilized plots during the years of the experiment and of which a large part may be considered to be removed with the crop, only included 180 and 245 kgm. per hectare. This is a very small percentage of the  $2\frac{1}{2}$  million kgm. of soil in the ploughed surface, 20 cm. deep. The saturation-concentration of  $P_2O_5$  in carbonic-acid-saturated water was very sensitive to these small additions of phosphates. In the experiments at Tystofte 0.48 mgm. of  $P_2O_5$  per liter of carbonic-acid-saturated water was found in the soil not fertilized with superphosphates, and 1.28 mgm. in the soil fertilized with superphosphates. In the experiments at *Lundby* there was a difference of 1.08 mgm. of  $P_2O_5$  between

Chili-salt-peter and Chili-salt-peter-superphosphates and 0.32 mgm. between Chili-salt-peter and to make up for potassium Chili-salt-peter-fertilizer-superphosphates. It is worthy of note that in the last instance in spite of the comparatively small difference in saturation-concentration of  $P_2O_5$ , the difference in speed of mannite-decomposition was great. This condition might indicate that mannite-decomposition expresses the assimilability of phosphoric acid in the soil both for the microorganisms in question and for the plant growth better than saturation-concentration expresses it. The condition found in sandy soil from *Blaahøj*, may be said to point in the same direction. This soil, which was probably the most phosphoric-acid-requiring soil of all these tested in these investigations, possessed a very slight power to break down mannite, while its saturation-concentration of  $P_2O_5$  was comparatively high (0.87 mgm.).

This investigation, as well as the investigations on the variation in the power of mineral soils to break down mannite, seems to show that comparative investigations of the relation of mannite-decomposition to the soil's content of readily soluble phosphoric-acid-combinations can only be made on *non-lime-requiring soils*. In the case of lime-requiring soils it will be necessary to add an amount of carbonate of lime sufficient for a maximum decomposition-speed before the decomposition experiments are begun.

The results of investigations with "uninoculated" cultures point in the same direction as the results of investigations with the "inoculated" but do not express as sharply the difference in the soil's content of readily assimilable phosphoric-acid-combinations as these.

The general relation between the reaction of the soil and the solubility of the phosphoric-acid-combinations in the soil is shown in the first part of table 6 which does not include the soils (cf. p. 350-351 drawn from various experiments with phosphoric acid fertilizers and the soil samples from Seeland and the Faroe Islands, which differed greatly from the ordinary types of Danish soils. The second part of table 6 shows the relations between the reaction, the azotobacter development of the soil in the azotobacter test, and its content of lime and magnesia in combinations soluble in ammonium chloride. In this report only the Seeland and Faroe Islands soils are omitted. Of these the two from Seeland showed a very large magnesia-content as compared with the Danish soils (see table 2).

The content of phosphoric acid in combinations soluble in muriatic acid, did not differ markedly within the single reaction groups.

There were much greater differences in the saturation-concentration of  $P_2O_5$  in carbonic-acid-extracts.

Among the loamy soils the saturation concentration of  $P_2O_5$  is very small in soil with acid reaction and the variations are also comparatively small (0.17-1.14 mgm.); in the neutral lime requiring soils without azotobacter development, the content of phosphoric acid in the carbonic acid extracts is, on the average, much greater although still small and again with compara-

TABLE 6  
*Relation of the solubility of phosphoric-acid-combinations in mineral soils, to their reaction and to azotobacter and content of lime-and-magnesia-combinations soluble in ammonium chloride*

source: the author's own observations

REACTION AND BASICITY	P <sub>2</sub> O <sub>5</sub> IN COMBINATIONS SOLUBLE IN MINERAL ACID						P <sub>2</sub> O <sub>5</sub> PER LITER OF CARBONIC-ACID-SATURATED WATER (SATURATION CONCENTRATION)			CaO SOLUBLE IN AMMONIUM CHLORIDE			MgO SOLUBLE IN AMMONIUM CHLORIDE								
	NUMBER OF SOILS						Average			Average			NUMBER OF SOILS								
	Least			Greatest			Least			Greatest			Least			Greatest			Average		
		per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Loamy soils																					
Acid.....	9	0.063	0.137	0.090	0.17	1.14	0.49	8	0.066	0.183	0.133	7	0.015	0.033	0.024						
Neutral without Azotobacter development.....	7	0.058	0.134	0.098	0.54	2.22	0.92	7	0.146	0.330	0.241	7	0.027	0.035	0.030						
Neutral with Azotobacter development.....	12	0.051	0.144	0.091	0.34	8.70	2.86	12	0.125	0.390	0.244	12	0.018	0.065	0.031						
Alkaline.....	13	0.061	0.177	0.092	0.46	10.0	2.71	12	0.282	1.110	0.534	12	0.015	0.032	0.023						
Without Azotobacter (lime-requiring soils).....	16	0.063	0.137	0.093	0.17	2.22	0.68	15	0.066	0.330	0.183	14	0.015	0.035	0.027						
With Azotobacter (non-lime-requiring soils).....	25	0.056	0.177	0.091	0.34	10.0	2.78	24	0.180	1.110	0.389	24	0.015	0.065	0.027						
Sandy soils																					
Acid.....	9	0.021	0.117	0.070	0.24	1.80	1.003	8	0.023	0.353	0.141	8	0.004	0.028	0.014						
Neutral without Azotobacter development.....	8	0.064	0.123	0.086	0.64	2.72	1.694	7	0.112	0.498	0.236	7	0.014	0.029	0.022						
Neutral with Azotobacter development.....	9	0.048	0.098	0.072	0.74	4.34	1.907	9	0.120	0.308	0.220	9	0.008	0.042	0.021						
Alkaline.....	10	0.054	0.150	0.100	1.55	12.24	4.013	10	0.332	0.924	0.575	10	0.012	0.029	0.021						
Without Azotobacter (lime-requiring soils).....	17	0.021	0.123	0.077	0.24	2.72	1.328	15	0.023	0.498	0.185	15	0.004	0.029	0.018						
With Azotobacter (non-lime-requiring soils).....	19	0.048	0.150	0.087	0.74	12.24	3.015	19	0.120	0.924	0.407	19	0.008	0.042	0.021						

All soils															
Acid.....	18	0.021	0.137	0.080	0.17	1.80	0.75	16	0.023	0.353	0.137	15	0.004	0.033	0.019
Neutral without Azotobacter development.....	15	0.038	0.134	0.091	0.54	2.72	1.33	14	0.112	0.498	0.238	14	0.014	0.035	0.026
Neutral with Azotobacter development.....	21	0.048	0.144	0.083	0.34	8.70	2.38	21	0.120	0.390	0.234	21	0.008	0.065	0.022
Alkaline.....	23	0.054	0.177	0.096	0.46	12.24	3.28	22	0.282	1.110	0.553	22	0.012	0.032	0.022
Without Azotobacter (lime-requiring soils).....	33	0.021	0.137	0.085	0.17	2.72	1.01	30	0.023	0.498	0.184	29	0.004	0.035	0.022
With Azotobacter (non-lime-requiring soils).....	44	0.048	0.177	0.089	0.34	12.24	2.88	43	0.120	1.110	0.397	43	0.008	0.065	0.024

\* 100 per cent = weight of air-dry soil.

\* 100 per cent = weight of air-dry soil.

tively small variations; in the neutral, non-lime-requiring soils with azotobacter development, the saturation concentration of  $P_2O_5$  is on the average six times as great as in the acid soils and about three times as great as in the neutral soils without azotobacter development. The soils with strong alkaline reaction are very similar to the neutral non-lime-requiring soils with azotobacter development. If the material is classified as lime-requiring and non-lime-requiring soils as in table 6, it will be seen that the saturation concentration of  $P_2O_5$  is about four times as great in the non-lime-requiring soils as in the lime-requiring soils. Another condition worthy of note is the fact that variations in the content of phosphoric acid in the extracts are much greater in the non-lime-requiring than in the lime-requiring soils. This indicates that *a certain lime-content is necessary for the presence of any considerable amount of phosphoric-acid-combinations soluble in carbonic acid*. This fact may also be seen by comparing (table 6) the reaction of the soil and its content of lime soluble in ammonium chloride. Sandy soils revealed, on the whole, the same condition as loamy soils. It should be noted here that the saturation-concentration of  $P_2O_5$  was, on the average, twice as large in both groups of lime-requiring soils as in the loamy soils. This may possibly express the fact that sandy soils are as a rule less prone to absorb phosphoric acid than loamy soils. The variation in the saturation-concentration of  $P_2O_5$  in the carbonic-acid-extracts even in sandy soils was rather small in the two lime-requiring groups and much less than in the two non-lime-requiring groups in which the phosphoric-acid-content in the carbonic-acid-extracts was about  $2\frac{1}{2}$  times as great as in the lime-requiring soils.

In consideration of the small number of soils and the very great variation within these groups in the content of phosphoric acid in the extracts, the very great difference present in the saturation-concentration in the two non-lime-requiring groups of soil in sandy soil may be assumed to rest to a certain extent on chance. Of all the soils examined, within the group of alkaline sandy soils, soil no. 89 has shown the largest amount of phosphoric-acid-combinations in carbonic-acid-extracts (12.2 mgm.  $P_2O_5$  per liter).

The extremely small content of phosphoric-acid-combinations soluble in carbonic acid found in field soils with strong acid reaction, and the comparatively small variations in this content agree very well with the results of investigations made by C. W. Stoddart (7) in the United States and M. Weibull (8) in Sweden by which it was demonstrated that field soils with an acid reaction are almost always deficient in phosphoric acid. In general, the results indicate that the reaction of the soil and its buffer content determine very decidedly not only the immediate content of easily soluble phosphoric-acid-combinations in the soil but also its power to transform phosphates added into combinations difficultly soluble.

Three of the soil samples investigated (18, 29 and 75) were drawn from soils known to be very highly fertilized. They came from farms where for many years a large number of cattle have been fattened for market on a very rich

diet of oil cakes and cereals. Table 2 shows that these soil samples, both as regards the absolute content of phosphoric acid and the saturation-concentration of  $P_2O_5$  in the carbonic-acid-extracts, were the richest in phosphoric acid within the reaction groups in question. Soil 75 was lime-requiring. Possibly that is why the saturation-concentration of  $P_2O_5$  is much less than in the other two soils; but within the group of neutral soils without azotobacter development it shows the greatest content of phosphoric acid in carbonic-acid-extracts.

Investigations of the content of magnesia-combinations soluble in ammonium chloride showed with a very few exceptions (soil 36 from Vejlø, 38 and 39 from Seeland), that this is small and as a rule much smaller than the content of lime soluble in ammonium chloride. It was impossible to show a closer relation between the reaction of the soil and the content of magnesia similar to that which had been found for lime by the author both in this investigation and in earlier investigations (2).

#### SUMMARY OF THE MAIN RESULTS

1. Observations seemed to show that important differences exist in the ability of various soils to decompose mannite.
2. A method for investigations of this kind was worked out.
3. An investigation was made of the variation in the mannite-decomposition power of soils expressed in the differences in speed of mannite decomposition.
4. Both "*uninoculated and inoculated*" cultures were used, in order to determine to what extent the variations found depended on differences in the chemical or microbiological nature of the soil. As a rule mannite-decomposition takes place more quickly in inoculated than in uninoculated cultures, but this difference is comparatively small, and the differences in the power of soil to decompose mannite depend primarily on the differences in the chemical condition of the soil.
5. A definite relation seemed to exist between the reaction of the soil (particularly its buffer action in the proximity of the neutral point measured by the author's azotobacter test) and its power of mannite decomposition.
6. No connection could be shown between the soil's content of phosphoric acid in muriatic-acid-soluble combinations, and its power to decompose mannite. On the other hand, experiments with *non-lime-requiring* soils proved by the azotobacter test to develop azotobacter showed that those among them having the greatest saturation-concentration of  $P_2O_5$  in carbonic-acid-extracts possessed, as a rule, the greatest power to decompose mannite.
7. A study of chemical factors showed that the content of basic, buffer-acting lime-compounds and of easily soluble phosphoric acid compounds determine the speed of mannite-decomposition.

The results seem to indicate that a determination of the speed of mannite-decomposition in non-lime-requiring soils, gives information as to the soil's content of easily soluble phosphoric-acid compounds readily assimilated by plants in the same way as the author's azotobacter test expresses the need of the soil for lime. Further investigations are desirable.

8. A survey was made of the relations between the reaction of the soil and its content of phosphoric acid in muriatic-acid-soluble-combinations, the saturation-concentration of phosphoric acid in carbonic-acid-extract, and of the content of lime and magnesia in ammonium-chloride-soluble combinations.

N.B. After this paper was finished a special investigation showed that the method used in determining the content of organic matter could be made more complete. The method is therefore being subjected to careful experimental tests and the results will be published in a short time.

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## DETERMINATION OF ORGANIC MATTER IN DECOMPOSITION EXPERIMENTS WITH SOIL

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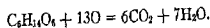
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In making decomposition experiments in soil, the determination of soluble organic matter in soil extracts was applied for the first time, so far as is known, by one of the authors (1) of the present paper in his test for mannite-decomposition in soil. In the investigation, soil was mixed with 2 per cent of mannite and the progress of decomposition tested at stated intervals by determining the amount of soluble organic substances present due to the addition of mannite in an aqueous filtrated extract.

The method employed is described in full in the paper referred to and is based on the same principle as that commonly used in determining the amount of organic matter in drinking water. A portion of the filtrate to which sulfuric acid has been added is heated with a surplus of very dilute, about 0.02 N potassium permanganate, after which the amount of potassium permanganate necessary to oxidize the organic substances present is determined in the usual way with the help of oxalic acid.

It soon became apparent that the usual 10-minute decomposition-period used in testing water was insufficient to oxidize completely the maximum amount of organic matter (about 8 mgm. mannite) in the given portions of the soil extracts. Portions of 1-10 mgm. mannite dissolved in distilled water were measured off. To each was added 3 cc. dilute sulfuric acid, 50 cc. .02 N potassium permanganate solution and enough distilled water to make 63 cc. In each case the  $\text{KMnO}_4$  concentration was the same. A special test showed the necessity of extending the decomposition period to 20 minutes and allowing oxidation to take place at not less than 80°C. This temperature was attained by plunging the beaker in which the reaction was taking place into boiling water. It was then possible to obtain both constant results and an entirely satisfactory agreement between the results of the replicate determinations.

The author later attempted a direct determination of mannite in the soil extracts. The results with the above method had not been wholly satisfactory and did not agree quantitatively with the equation:



According to this equation, 71.4 cc. of .02 N potassium permanganate solution is required to oxidize 10 mgm. mannite, whereas in the experiment made only 36.4 cc. was used. A corresponding oxidation of but 1 mgm. mannite required 7.3 cc. permanganate or 102 per cent of the amount computed. Table 1 indicates that with increasing amounts of mannite the actual amount of permanganate used becomes steadily less than the computed amount.

This investigation seems to indicate very clearly that a considerable surplus of potassium



permanganate is necessary to oxidize mannite completely to carbonic acid and water. Decomposition occurred regularly as long as no more than 25–30 cc. permanganate solution was used with 4–5 mgm. mannite. The difference between the actual and the computed values at this point was still comparatively slight; i.e., 17–19 per cent. When greater quantities of mannite were used the difference was very considerable; with 6 mgm. about 30 per cent; with 8 mgm., about 41 per cent; and with 10 mgm., about 50 per cent.<sup>1</sup> In another experiment, 50 and 100 cc. permanganate solution were used to oxidize 10 mgm. mannite. Here the amounts of permanganate were used respectively 53 and 78 per cent of those computed.

These investigations suggested that more complete data on the relation between the amounts of mannite and potassium permanganate might make it possible to work out a quantitative method.

TABLE I  
*Relation between the amounts of mannite and potassium permanganate used*

MANNITE	AMOUNT OF 0.02 N $\text{KMnO}_4$ SOLUTION USED				
	Determinations			Average corrected for check	Relation to the amount computed
	a	b	Average		
mgm.	cc.	cc.	cc.	cc.	per cent
0	1.3	1.4	1.4		
1	8.4	8.9	8.7	7.3	102.2
2	15.1	15.2	15.2	13.8	96.7
3	19.9	20.5	20.2	18.8	87.8
4	24.8	25.6	25.2	23.8	83.4
5	30.6	30.0	30.3	28.9	81.0
6	31.9	32.0	32.0	30.6	71.5
7	32.7	33.3	33.0	31.6	63.3
8	34.8	34.9	34.9	33.5	58.7
9	35.9	36.7	36.3	34.9	54.3
10	37.4	38.0	37.7	36.3	50.9

A few preliminary investigations on the effect of lengthening the heating period and the addition of various substances which might be supposed to have a catalysing effect and so further oxidation, showed that the object was not to be attained in this way. The effect of a 0.1 N and a 0.05 N solution instead of the 0.02 N potassium permanganate was tested. The amount of the 0.02 N solution used was not increased because it seemed desirable to avoid using unreasonably large amounts of titrating fluid.

It was then found that 0.1 N  $\text{KMnO}_4$  gave unreliable results. Only half as much potassium permanganate solution was used in the separate portions after which the addition of distilled water brought the total amount of liquid in each up to 63 cc.

<sup>1</sup> Earlier investigations by the author on the use of  $\text{KMnO}_4$  in the oxidation of pure mannite (1) showed that 10 mgm. mannite required 43 cc. 0.02 N potassium permanganate solution. In this investigation, however, liquid portions containing 6 mgm. mannite were used, after which the results for 10 mgm. were computed. This explains the lack of agreement between the two investigations.

Table 2 shows that small amounts of mannite (1 and 2 mgm.) required more of the 0.1 *N* permanganate solution than the *theoretical* amount. The cause of this has not yet been determined but titrating back again with a strong permanganate solution showed a clear change to be difficult due to the precipitation of  $MnO_2$ . With 0.02 *N* solution instead of 0.1 *N* on titrating back again, the agreement between the theoretical and actual values was much better. Expressed in percentage, the relation diminished gradually from 109.5 (1 mgm. of mannite).

A series of test with 0.05 *N*  $KMnO_4$  in 50-cc. potassium permanganate portions was made, using 0.02 *N* solution for titrating back.

Table 3 shows some of the results. Here again the figure for the smallest amount of mannite used is too high, but the difference between the actual and

TABLE 2  
*Relation between the amounts of mannite and potassium permanganate used*

MANNITE	AMOUNT OF 0.1 <i>N</i> $KMnO_4$ USED (SAME SOLUTION USED FOR TITRATING BACK)				
	Determinations			Average corrected for check	Relation to the amount computed
	a	b	Average		
mgm.	cc.	cc.	cc.	cc.	per cent
0	0.2	0.1	0.2		
1	2.7	2.8	2.8	2.6	179.6
2	3.8	4.1	4.0	3.8	133.1
3	4.9	4.7	4.8	4.6	107.4
4	6.2	6.2	6.2	6.0	105.1
5	7.5	7.6	7.6	7.4	103.7
6	8.7	8.9	8.8	8.6	100.4
7	10.4	10.3	10.4	10.2	102.1
8	11.2	11.0	11.1	10.9	95.3
9	12.3	11.8	12.1	11.9	92.6
10	13.3	13.0	13.2	13.0	91.1

computed values is only comparatively small, and so far as the method is concerned it is of no importance. In any case the use of only about 7 cc. potassium permanganate will indicate that the decomposition is approximately complete, and at that point it is immaterial if the amount used is 1 cc. larger. A content of 2-4 mgm. mannite showed practical agreement between the values found and computed, but when 8 mgm. mannite was present, which was about the maximum amount possible in the soil extracts examined,<sup>2</sup> the figure found is 7 per cent too low. It is therefore, better not to use quantities of the extract larger than those corresponding to a content of 4-5 mgm. mannite.

<sup>2</sup> The portions of soil extract measured off (10 cc.), correspond to 0.5 gm. moist soil (about 0.4 gm. air-dry). At the beginning of the experiment the soil contained 10 mgm. mannite per 0.5 gm. air-dry soil. The values actually found, then, are about 20 per cent lower than those computed. This relationship is of some importance in interpreting the results of earlier experiments.

By using 0.05 *N* potassium permanganate solution for the oxidizing agent, it is possible to determine the amount of mannite present in soils with a degree of accuracy sufficient for tests on the ability of soils to decompose mannite.

The modified method of procedure is as follows:

Of the soil extracts prepared,<sup>1</sup> place an amount corresponding to about 0.25 gm. soil in a 400-cc. beaker with 50 cc. .05 *N* potassium permanganate solution and 3 cc. diluted sulfuric acid (6:100). Heat this for 20 minutes by plunging the beaker into boiling water. Add 50 cc. 0.05 *N* oxalic acid solution and titrate with 0.02 *N* potassium permanganate solution.

The very detailed calculation necessary if the permanganate solution and the oxalic acid are not exactly 0.02 *N* may be avoided by using a check in which all errors in method are included. Then only one correction for the 0.02 *N* potassium permanganate solution is necessary.

TABLE 3  
*Relation between the amounts of mannite and potassium permanganate used*

MANNITE	AMOUNT OF 0.05 <i>N</i> KMnO <sub>4</sub> USED (0.02 <i>N</i> SOLUTION USED FOR TITRATING BACK)				
	Determinations			Average corrected for check	Relation to the amount computed
	a	b	Average		
<i>mgm.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>	<i>per cent</i>
0	1.6	1.6	1.6		
1	9.8	8.7	9.3	7.7	107.8
2	16.4	15.5	16.0	14.4	100.9
3	23.2	22.6	22.9	21.3	99.5
4	29.5	30.7	30.1	28.5	99.8
5	35.8	35.9	35.9	34.3	96.1
6	41.8	41.7	41.8	40.2	93.9
7	48.5	47.8	48.2	46.6	93.3
8	55.4	53.7	54.6	53.0	92.8
9	62.2	61.7	62.0	60.4	94.0
10	65.2	65.9	65.6	64.0	89.7

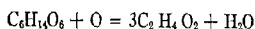
Table 1 shows that the values obtained (taken both absolutely and relatively) from the method used in earlier investigations on the ability of soil to decompose mannite, were too low in the first stages of mannite decomposition. This does not, however, prevent a clear interpretation of results. A consumption of 35-40 cc. of 0.02 *N* potassium permanganate solution under the conditions of those investigations, must be considered an expression of the fact that only a slight or no decomposition of the mannite contained in the soil took place. A consumption of more than 30 cc. is an expression of the fact that the larger part of the mannite is still present in the soil. At the close of 2-3 periods,

<sup>1</sup> On the preparation of the extract see the author's (1) earlier article. To reduce the possibility of errors in analysis it is recommended to use 200 cc. water in preparing the soil extracts instead of 100 cc. In that case, 10 cc. of the filtrate should be measured off for analysis. If the relationship between soil and water used hitherto is kept, only 5 cc. of the extract is used.

(10-15 days), a content of organic matter present corresponding to more than 20-25 cc. expresses, in every case, the fact that mannite decomposition occurred very slowly.

Although, it is probable that no other conclusions would have been reached by using a more perfect method in determining the content of organic matter in soil extracts, yet it is evident that the results would appear more certain if expressed in values more nearly correct even from an absolute point of view. This study has revealed a method by which this is possible.

In connection with the investigations so far discussed, a few other experiments were made. The object of these was to explain the peculiar fact that only when a *considerable surplus* of  $\text{KMnO}_4$  is used does complete oxidation of mannite to carbon dioxide and water take place. When this surplus is not present a very considerable amount of acetic acid, a substance not affected by permanganate, is formed. In such experiments 100 mgm. mannite was oxidized with 500 cc. of 0.02 *N*  $\text{KMnO}_4$ . When the precipitated  $\text{MnO}_2$  has been filtered out, the filtrate is neutralized with carbonate of soda, evaporated, led over into a fractionally graded retort, phosphoric acid added and then distilled. It was very easy to make a qualitative determination of the presence of acetic acid. A titration of a part of the distillate will show considerable acidity. That a very greatly reduced consumption of  $\text{KMnO}_4$  appears when mannite is oxidized to acetic acid is readily explainable. If extreme oxidation is carried on according to the equation:



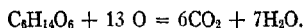
only one-thirteenth of the amount of permanganate necessary when a complete oxidation to carbon dioxide and water takes place, will be used.

In this connection we may add that direct experiments have shown that *n*-butyric acid is not affected by potassium permanganate. This fact is important for we may suppose that in mannite decomposition acetic acid and *n*-butyric acid as well as carbonic acid are formed. Lactic acid and isobutyl-alcohol, two products also resulting from mannite decomposition are readily oxidized by  $\text{KMnO}_4$ . According to our present knowledge of the course of decomposition these two latter substances will appear in much smaller quantities, only slightly affecting the amount of permanganate used. This amount depends for the most part on the amount of *undecomposed mannite*. Since direct mannite determinations are difficult and since, to the author's knowledge, there is no exact method for a quantitative determination of such small quantities as these experiments deal with, this fact is of great importance in the interpretation of the experimental method worked out here.

#### SUMMARY

Investigations of the possibility of a direct determination of mannite in soil extracts showed that the method hitherto used in determining organic sub-

stances in the soil extracts is not entirely satisfactory, for the oxidation of the greatest amount of mannite able to appear did not occur quantitatively according to the equation:



This result was made the subject of a special investigation. It was found that a *very considerable surplus of potassium permanganate* is necessary for a quantitative oxidation.

A modified method for the determination of organic matter is recommended.

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## REVERSION OF ACID PHOSPHATE IN ACID SOILS

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In an attempt to gain some knowledge of the fate of superphosphate when applied to acid soils with and without the addition of limestone, a series of mixtures of some samples of acid soil, superphosphate and limestone were made, allowed to stand for a month, and then analyzed for "available" phosphoric acid.

### MATERIALS USED

Two of the most acid soils encountered by this station were used, viz., soil X and soil Y.

Soil X is a brown sandy loam, with a lime requirement of 3713 pounds of  $\text{CaCO}_3$  per acre (Veitch).

Soil Y is a dark grey, very light, peaty soil containing a large amount of organic matter with a lime requirement of 3740 lbs.  $\text{CaCO}_3$  per acre. This soil had an abnormally high water-holding capacity of about 200 per cent.

Soil X contained 0.21 per cent lime and soil Y 0.34 per cent.

The superphosphate used contained 14.37 per cent water-soluble and 14.94 per cent citric-acid-soluble phosphoric oxide.

Two grades of limestone were used, A which passed a 1 mm. mesh sieve and contained 94.4 per cent calcium carbonate, and B which passed a 3 mm. mesh sieve, but not a 1 mm. mesh sieve and contained 74.3 per cent calcium carbonate.

The acidity of the soils was calculated to be equivalent to 1.5 gm.  $\text{CaCO}_3$  per kilogram of soil which is equivalent to 2.1 gm. of the "medium" or 1.6 gm. of the "fine" limestone. The acidity of the superphosphate was found to be equivalent to 576 gm. of the "medium" or 428 gm. of the "fine" limestone per kilogram.

### MIXTURES MADE

Mixtures were made of 1 kilogram of air-dry soil, 25 gm. of superphosphate and varying amounts of limestone. After being moistened, the mixtures were stored in the dark in rubber-ringed mason jars for a month.

The two series of mixtures were made equally damp by adding water to 70 per cent of their capacity.

After a month the equivalent of 100 gm. of air-dry soil was weighed out from each mixture and the "available" phosphoric oxide extracted by the 2-per-cent citric acid method and determined by the volumetric molybdate method.

*Mixtures made with Soil X and "fine" limestone A*

1. 1 kgm. soil with 25 gm. superphosphate.
2. Limestone to satisfy the requirement of 1 kgm. of soil and 25 gm. superphosphate was mixed with 25 gm. of superphosphate which was then added to 1 kgm. of soil.
3. 1 kgm. soil with limestone calculated to satisfy the requirement of the soil and 25 gm. of superphosphate and then 25 gm. superphosphate mixed in.
4. 1 kgm. soil with 25 gm. superphosphate and then the limestone to satisfy the requirement of both soil and superphosphate mixed in.
5. 25 gm. superphosphate mixed with limestone to satisfy its requirement and then mixed with 1 kgm. of soil.
6. 1 kgm. of soil mixed with the limestone requirement of 25 gm. superphosphate and then with 25 gm. of superphosphate.

*Mixtures made with Soil X and "medium" limestone B*

7. Same as 2.
8. Same as 4.

*Mixtures made with Soil Y and "fine" limestone A*

9. Same as 1.
10. Same as 2.
11. Same as 3.
12. Same as 4.

*Mixtures made with Soil Y and "medium" limestone B*

13. Same as 6.

TABLE I  
Percentage reversion of acid phosphate in acid soils

MIXTURE NUMBER	PHOSPHORIC OXIDE		REVERSION <i>per cent</i>
	In original mixture <i>per cent</i>	After 30 days <i>per cent</i>	
1*	0.364	0.275	24.46
2	0.360	0.270	25.00
3	0.360	0.270	25.00
4	0.360	0.270	25.00
5	0.360	0.260	27.89
6	0.360	0.255	29.29
7	0.358	0.245	31.68
8	0.358	0.250	30.29
9	0.364	0.145	60.16
10	0.360	0.140	61.11
11	0.360	0.140	61.11
12	0.360	0.145	59.72
13	0.359	0.135	62.43

\* These two mixtures were unlimed.

From these figures it would appear that there is no actual benefit, as far as the availability of the superphosphate is concerned, to be had from mixing limestone with superphosphate or applying them to the soil separately even in different order.

With both soils the limed mixtures lost slightly more than the unlimed mixture except for mixture 12. Those mixtures limed with the coarser grade limestone seem, however, to have reverted most. It might be thought that the coarser grade limestone contained impurities that had made it more resistant to grinding and that these impurities caused the greater reversion of the phosphate. Analysis showed, however, that whereas the fine grade contained 4.53 per cent of iron oxide the medium grade contained only 0.26 per cent. A comparison of analyses made of two limestones—sieved into three grades after grinding—showed the amount of iron oxide to be greatest in the fine grade and least in the coarse. The lime content was just the opposite, the coarsest grades had the highest amount.

TABLE 2  
*Partial composition of untreated soils*

SOIL	Fe <sub>2</sub> O <sub>3</sub>	Al <sub>2</sub> O <sub>3</sub>	P <sub>2</sub> O <sub>5</sub>	CaO	VEITCH LIME REQUIREMENT
X	1.58	1.65	0.06	0.21	3713
Y	5.67	9.79	0.20	0.34	3740

How far the conditions of the experiment have affected the results is not known, but these figures certainly do not show any benefit a month afterwards from liming the soil when the amount of phosphate reverted is taken as the criterion. There is a large difference in the percentage of reversion in the two soils, although their lime requirements are the same when measured by the Veitch Method. The lime requirements of the two soils also agreed very closely when measured by the method of shaking the soil with alcoholic potassium thiocyanate and titrating the red color obtained with caustic soda. This method shows that the solubility of the iron in each soil is practically the same, and the different rates of reversion in the two cases are therefore, not due to a greater proportion of soluble iron in the one case.

A subsequent analysis showed that the soil having the most total iron caused the most reversion. Unfortunately it also had the most alumina as shown in table 2. Soil Y containing the greatest amount of iron oxide and alumina showed the greatest reversion of the phosphate despite the addition of lime. Comparing the amounts of these substances in the two soils, the reversion is not as great in Y as might be expected when the reversion in X and its composition are considered.





## AVAILABILITY OF ADSORBED PHOSPHORUS

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There is no question but that both the positive and negative ions of various salt solutions are adsorbed by soil colloids. Work by Gordon and Starkey (3) and by Lichtenwalner, Flenner and Gordon (1) has demonstrated this fact. Also, these studies showed that the salts adsorbed by the soil colloids of alumina and ferric oxide are only about one-third removed by the usual leaching process. This raised the question, whether the salts, which are adsorbed by these colloids and which cannot be dislodged by washing, are available to the plant when needed or are they forever locked in the soil in an unavailable form? The present investigation was taken up to throw some light on this question.

Colloidal ferric oxide and alumina were prepared as previously described. The colloids were allowed to suffer their maximum adsorption in a 0.05 *N* potassium acid phosphate solution. They were then subjected to washings until the filtrate gave no test for the adsorbed phosphate. On analysis, these gels were found to contain about one-third of their original phosphate.

Sweet potato plants were used because of the ease with which the roots might be divided between the two jars used in each experiment. Seed potatoes were sprouted in sand. Sprouts about six inches long were transferred to double jars consisting of two 250-cc. glass jars firmly bound side by side, so they retained the same position relative to each other. The numbers 1-8 refer to double jars and 1a and 1b, 2a and 2b, etc. refer to the individual jars. Nutritive solutions were prepared and used as recommended by the National Research Council (2). Two sets of four double jars filled with white quartz sand were used. Colloidal ferric oxide which contained adsorbed unleachable phosphate was thoroughly mixed with the sand in 2b, 3b and 4b and colloidal alumina with adsorbed unleachable phosphate was mixed with jars 6b, 7b and 8b. The seedlings were weighed and one planted in each double jar. Half of the roots were placed in one side of the double jar and half in the other.

Jars 1a and 2a received distilled water; jars 1b 2b, 3a and 3b, a complete fertilizer solution minus phosphorus; jar 4a, a complete nutrient solution while 4b had a complete nutrient solution minus phosphorus. Jars 5-8 were treated in the same way.

After the plants had grown for eight weeks it was found that the roots filled the entire glass jars. The plants were then taken up, weighed and analyzed for phosphorus. The data are given in table 1.

Plant 1 ceased to grow when planted in jar 1 and showed a slight decrease in phosphorus content. Plant 5 also lost in phosphorus content, but in-

creased slightly in weight. Since neither of these double jars contained phosphorus, the migration of the phosphorus would naturally be outward. Also, the lack of phosphorus seemed to almost completely inhibit the growth of the plant. Plants 2 and 6 showed a marked increase in the weight of the plant and in phosphorus content. These plants had no source for phosphorus except adsorbed unleachable phosphorus. Plants 3 and 7 made a slight gain in phosphorus over 2 and 6 respectively, although they had access to

TABLE 1  
*Growth of plants and phosphorus content*

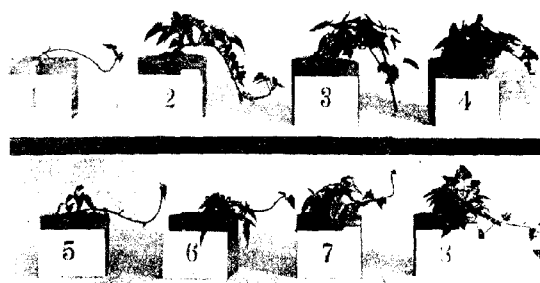
PLANT NUMBER	WEIGHT OF ORIGINAL PLANT	WEIGHT OF MATURE PLANT	GAIN IN WEIGHT	P IN ORIGINAL PLANT	P IN MATURE PLANT	GAIN IN P CONTENT
	gm.	gm.	gm.	mgm.	mgm.	mgm.
<i>Ferric oxide colloid</i>						
1 (control)	1.70	1.70	0.00	1.00	0.9	-0.1
2	2.55	19.10	16.55	1.49	6.74	5.25
3	3.40	30.20	26.80	1.99	10.22	8.23
4	2.20	34.40	32.20	1.29	20.76	19.47
<i>Alumina colloid</i>						
5 (control)	3.10	6.40	3.30	1.82	1.65	-0.17
6	2.10	13.90	11.80	1.23	4.20	2.97
7	2.10	13.50	11.40	1.23	4.73	3.50
8	2.05	14.35	12.25	1.20	7.82	6.62

no more phosphorus. Plants 4 and 8 made a marked gain in both weight and phosphorus content. This would be expected since they had phosphorus in the nutrient solution in addition to the adsorbed phosphorus. At first thought this might seem to show that the plant could not get sufficient phosphorus for its natural growth from the unleachable adsorbed phosphorus yet the authors wish to do further work before making this statement.

This investigation has shown that phosphorus which has been adsorbed by soil colloids and which cannot be leached out by water is available for plant food.

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SWEET POTATO PLANTS AT COMPLETION OF EXPERIMENTS



## THE EFFECT OF DIFFERENT REACTIONS ON THE GROWTH AND CALCIUM CONTENT OF OATS AND WHEAT<sup>1</sup>

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In a previous investigation (2) the author reported the effect of different reactions on alfalfa and clover. This work was later extended to include the effect of different reactions on the growth and calcium content of oats and wheat. These plants were included, because of their importance as crops, and because of the lack of definite information along this line concerning them. The results of this investigation are reported in this paper.

It is generally recognized among agronomists that the cereals are less sensitive to acidity than most of the cultivated legumes. This is possibly due to the greater amount of basic material required for the normal growth of the legumes than for the cereals as suggested by Truog (15). Hartwell and Pember (5), (6) working with some of the cereals reported that there is a wide difference in the behavior of these plants toward acid soils. They found the order of sensitiveness to soil acidity, starting with the most sensitive, to be barley, wheat, oats and rye. When grown in solution cultures of varying degrees of acidity, they noted no appreciable difference in the behavior of these plants. They concluded that the presence of toxic aluminum salts and not acidity directly, caused the difference in behavior of the plants. They also reported (5) that acidity was more toxic to the growth of these plants than an equal alkalinity. Hoagland (7), Conner (3) and others later reported the reverse of this for barley, and Salter and McIlvaine (11) reported the reverse for wheat. The work of Conner (3), and Hartwell and Pember (5), (6) was largely concerned with the part played by aluminum in acid soils, which is probably an important factor in some cases.

Tarr and Noble (13) reported that wheat produced a maximum growth in solution cultures at a reaction of about pH 4. This does not seem to be exactly in accord with the results of other investigators (3), (5), (11).

The recent investigations by True (14) concerning the function of calcium in the nutrition of seedlings, and Truog (15), (16) concerning the feeding power of plants have shown the necessity of calcium as a nutritive element, as well as an amendment to acid media to induce a desirable assimilation of the other nutritive elements. A deficiency of calcium as a plant food in many acid soils has been reported by Shedd (12) MacIntire, (10), and others.

In general the data indicate that the cereals are less sensitive to acids than to alkalis, and also less sensitive to acids than most of the legumes. It

<sup>1</sup> Published with the permission of the Director of the Wisconsin Agricultural Experiment Station. The greenhouse work in connection with this investigation was done at the University of Wisconsin and the chemical analysis at the University of West Virginia.

The author wishes to express his appreciation for the helpful suggestions and criticisms tendered by Professors E. Truog and E. B. Fred.

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appears that wheat is more sensitive to acid media than oats, but no exact effect of reaction on these plants is given. The reaction of the medium in which the plants are grown seems to have a direct influence on the assimilation of the nutritive elements.

#### EXPERIMENTAL

The Kherson oat and Marquis wheat were used in this investigation. All the experiments were carried out in quartz cultures, using the same containers and methods of growing the plants as described in the previous publication (2). The nutrient solution consisted of Crone's normal solution plus  $\frac{1}{4}$  gm. of di-sodium phosphate per liter. The sodium phosphate was added to increase the buffer action of the solution. Varying amounts of dilute sulfuric acid and sodium carbonate were added to portions of this solution to obtain the different reactions of approximately pH 3, 4, 5, 6, 7, 8, 9 and 10. The

TABLE I  
*The growth and calcium oxide content of oats and wheat in sand cultures with Crone's nutrient solution at different reactions*

APPROXIMATE REACTION	OATS		WHEAT	
	Dry weight of 4 plants	Content of CaO	Dry weight of 4 plants	Content of CaO
pH	gm.	per cent	gm.	per cent
3.3	2.4		2.8	
3.9	4.4		2.8	
5.0	8.8	0.57	6.5	0.51
6.0	10.7		9.5	
6.9	10.0	0.52	9.4	0.52
7.8	5.3		5.1	
8.8	4.3	0.40	3.8	0.51
9.6	1.4		0.9	

acid was used to adjust the reactions in the acid range and the carbonate in the alkaline range. The solutions were renewed daily as previously described (1, 2) in order to keep the reactions as constant as possible.

The seedlings were allowed to grow at the different reactions for 2 months. The sand was then washed from the percolators and the photographs made of the entire plants. The plants were dried and the calcium determination made as previously described for alfalfa and clover (2). All the experiments were carried out in duplicate, and the results are reported as averages. Table I and plate 1 give the results and growth of the plants at the different reactions.

Reference to plants grown at the different reactions will be made by referring to pH 3, 4, 5, 6, 7, 8, 9 and 10 although the actual pH values may have been slightly more or less. The average pH values are given in table 1.

It will be noted from this table that the maximum growth of oats took place at about pH 6, while that of wheat took place at a slightly less acidity. The

oat plants produced a much better growth at pH 4 and 5 than did the wheat. There was a considerable retardation of both the oats and wheat at pH 3, 4, 9 and 10. The retardation of wheat was greater than that of oats. No stooling or branching of any of the plants took place at pH 3 and 10. One plant each of the oats and wheat was almost dead at pH 10. A decrease in acidity from pH 5 produced a decrease in the calcium content of the oats, but no appreciable difference in the calcium content of the wheat at the different reactions was found. Four determinations of calcium were made at the pH values indicated and the results represent the averages.

#### DISCUSSION

The better growth of the oats in the acid reactions than that of the wheat is possibly due to the greater normal acidity of the sap of the oat plants than that of the wheat. The normal acidity of the oat sap is about pH 5.6, while that of wheat is about pH 6.2 (4). The distinctly acid reaction of the oat sap, and the small amount of calcium required for its normal growth are no doubt important factors influencing the calcium in the plant. It is possible that the greater sodium content of the more alkaline solutions partially replaced the calcium in the oat plants with increase in alkalinity. Meyer (9) noted a decrease of calcium in the oat plant when grown on soils of high lime content and presumably less acid.

Since the sap of the wheat plant has about the same reaction as that of alfalfa and clover, and requires less than one-half the amount of calcium for its normal growth, the influences of reaction on the power of this plant to assimilate calcium would seem to be comparatively small, as was actually found in this investigation. On the other hand, a reaction more acid than the sap of the alfalfa and clover plants decidedly decreased the power of these plants to secure their necessary amount of calcium, as was shown in the previous investigation (2). The maximum growth of oats and wheat took place at a slightly acid reaction of pH 6 to 7, while that of alfalfa and clover took place at a neutral or slightly alkaline reaction of pH 7 to 8.

The results with wheat in this investigation do not agree exactly with those of Tarr and Noble (13) who reported that wheat produced a maximum growth in solution cultures at about pH 4. Salter and McIlvaine (11) reported a maximum growth of wheat at about pH 6, and of corn at about pH 5. Field observations indicate that wheat is less acid tolerant than corn. The different results obtained by Tarr and Noble (13) may be due to the composition of the nutrient solution used. It is very improbable that wheat will produce a maximum growth in soils at a reaction of pH 4.

There are undoubtedly other factors which affect the power of plants to assimilate the necessary nutritive elements, but it seems that the reaction of the medium exercises a very important influence. This possibly applies to all the plant nutrients as well as to calcium. The acidities which were injurious



to the oat and wheat plants in this investigation were less than that of many acid soils. The effect of acid soils on plant growth will be given in a later report.

#### SUMMARY AND CONCLUSIONS

Oat and wheat plants were grown for two months in quartz cultures at different reactions. The nutrient solutions were changed daily in order to keep the reactions as constant as possible. The results may be summarized as follows:

1. The maximum growth of the oat plants took place at about pH 6, and that of wheat at a slightly less acidity of pH 6 to 7.
2. The oats grew at pH 4 and 5 much better than did the wheat. In general the oats were less affected by acidity than the wheat.
3. The oats and wheat produced practically no growth at pH 3 and 10, which are near their critical reactions.
4. An increase in acidity or alkalinity from the range pH 6 to 7 produced a decrease in the growth of both the oat and wheat plants.
5. A decrease in acidity from pH 5 produced a decrease in the calcium content of the oat plants, but not of the wheat plants.
6. The acidities which were injurious to the growth of the oats and wheat in this investigation are no greater than that of many acid soils.

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## PLATE 1

FIG. 1. OATS GROWN AT DIFFERENT REACTIONS IN QUARTZ SAND WHICH WAS REMOVED  
FOR MAKING THE PHOTOGRAPH

The numbers represent the approximate pH values

FIG. 2. WHEAT GROWN AT DIFFERENT REACTIONS IN QUARTZ SAND WHICH WAS REMOVED  
FOR MAKING THE PHOTOGRAPH

The numbers represent the approximate pH values

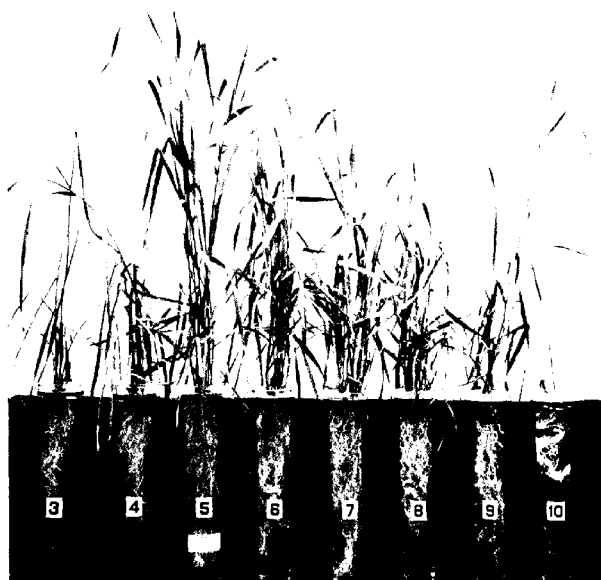


FIG. 1

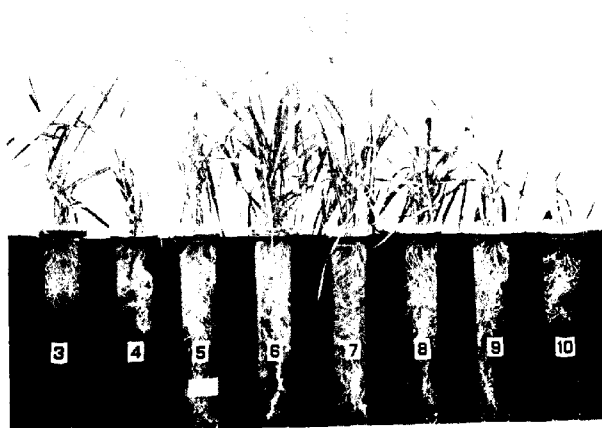


FIG. 2



# EFFECT OF ADSORPTION AND OTHER FACTORS ON CERTAIN PLANT FOOD CONSTITUTENTS OBTAINED IN THE DILUTE NITRIC ACID DIGESTION OF SOILS AND AN IMPROVEMENT FOR THEIR ESTIMATION<sup>1</sup>

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## INTRODUCTION

It is generally conceded that plant food exists in soils in two forms. One is often referred to as easily soluble, active or available, and consists of that smaller portion which plants can utilize for immediate needs as distinguished from the larger but less available reserve supply in the soil. The latter, of course, can be converted into the former by proper treatment of the soil. The distinction between the two forms and the use made of them by plants is well illustrated in the cultivation of virgin or old sod land in such crops as tobacco or potatoes for one or two seasons. Experience shows that this practice rapidly impoverishes the soil especially if the same crop is grown continuously without fertilization. This is probably due to the rapid removal of the more easily soluble plant food. The result is that the soil, although it may still contain an ample supply of total plant food and be free of diseases peculiar to these crops, nevertheless has to be restored somewhat to its virgin state before equivalent yields can be obtained. In other words, the more easily soluble plant food removed must be replaced. The problem, therefore, is to accomplish this as well as the natural agencies did before cultivation, and in less time, if possible.

## HISTORICAL

Numerous procedures have been used for the estimation of the easily soluble plant food in soils. The most popular is the weak acid digestion. The mineral acids, hydrochloric or nitric, are employed in this country whereas citric acid has found favor abroad.

Any laboratory method that might be proposed for this purpose is more or less arbitrary notwithstanding that results obtained by its use may be corroborated by field tests. For this reason, considerable discussion has arisen as to the value of any such method. Opponents contend that it is impossible to duplicate soil conditions in the laboratory, therefore no credence can be placed in such work. To support this they cite the fact that different methods often give widely different results on the same sample. Some even maintain that

<sup>1</sup> Published by permission of the Director of the Kentucky Agricultural Experiment Station.

<sup>2</sup> The writer desires to thank Dr. A. M. Peter, Head of the Department of Chemistry, for helpful criticism during this investigation.

all chemical methods, including those for the estimation of total plant food constituents, give no reliable data for indicating the productive capacity of soils. Other workers assert that some of the methods do give results of a certain value when properly interpreted. They cite the fact that their findings have often been verified in the field and for this reason they continue to employ them in their work.

It is not necessary to discuss the relative merits of these opposite views or to include references to same in the literature as they are very voluminous. For this reason, only those which refer to certain matters discussed here are mentioned.

The most widely used methods in this country are those based on digesting the soil either in 0.2 *N* hydrochloric or nitric acid for a limited time at a definite temperature. The former was adopted by the Association of Official Agricultural Chemists (2) as a provisional method for easily soluble phosphate in soils. The literature at hand shows that the first work in this country with 0.2 *N* nitric acid as a solvent was done by Dr. A. M. Peter, at this Station (1, p. 77-80). This method was later recommended as a substitute for the 0.2 *N* hydrochloric acid method (3). It should be stated, however, that the method used and recommended at that time differs materially in some respects from the procedure as carried on in this work.

The last committee appointed by this Association for the revision of methods has failed, for some unaccountable reason, to include any method for the above separation, consequently none appears in the latest revision of "Methods of Analysis" (4).

Russell and Prescott (6) have made very extensive experiments on the determination of phosphorus obtained in the weak acid digestion of soils. Their work includes only this element and consists of a study of its adsorption by the soil and the effect of time of digestion and different strengths of various acids on the results obtained. They show that a short digestion with some acids, especially nitric acid not exceeding a certain strength, puts more phosphorus into solution than does one for a longer time. This they prove to be due to adsorption. On account of the different methods of procedure, different strengths of acid employed and the limited number of soils used in their work, their conclusions regarding the behavior of nitric acid have been only partly confirmed here. An excellent summary of some previous investigations on soil adsorption is given by these writers and in a résumé by Prescott (5).

During the course of some experiments on the digestion of soils with 0.2 *N* nitric acid early in 1916, the writer observed that adsorption apparently had considerable influence on the results obtained especially in the phosphorus determinations. These experiments were continued so as to include a large number of different types of soil and the estimation of elements other than phosphorus.

It was soon observed that short periods of digestion, for example, 5 minutes, showed as much or more phosphorus in solution in some soils as the regular 5 hour period. The short digestion also gave nearly as much potassium and calcium. All soils, however, did not behave the same in this respect. The results apparently showed that the effect of adsorption in some soils in the long digestion was a factor which could not be disregarded. As a result of this work, a short qualitative method for testing for easily soluble phosphate in soils was devised and published (7). In that paper, experiments are recorded which clearly show that the short 0.2 *N* nitric acid digestion is valuable for indicating the needs of a soil for phosphorus and in the present one others are given which extend its application to other plant-food constituents.

#### METHODS

##### *Fifth normal nitric acid method*

The procedure was to digest the air dried soil of 2 mm. or less in fineness in the proportion of 1 gm. of soil to 10 cc. of 0.2 *N* HNO<sub>3</sub> for 5 hours at room temperature, shaking every 30 minutes. If the sample contained carbonate

correspondingly stronger acid than the above was used so as to neutralize it without increasing the volume. After the digestion period the mixture was shaken again and poured on a large folded filter. The first 100 cc. of filtrate was poured back twice. The filtration usually required from 20-30 minutes when 120 gm. of soil was used. An aliquot was evaporated to dryness. More  $\text{HNO}_3$  was added to oxidize organic matter, the solution was again evaporated and the last trace of  $\text{HNO}_3$  eliminated by evaporation with  $\text{HCl}$ . The residue was dried on the steam bath to dehydrate  $\text{SiO}_2$ , taken up with  $\text{HCl}$  and  $\text{H}_2\text{O}$ , filtered and made to a definite volume. Separate aliquots corresponding to 40 gm. of soil were used for the determination of phosphorus and potassium and to 5 or 10 gm. for calcium. For those soils which contained large amounts of phosphorus, the aliquot represented 5 or 10 gm. of soil.

Phosphorus was determined with ammonium molybdate by precipitating at  $40^\circ\text{C}$ . in a small volume and allowing the yellow precipitate to stand at that temperature for an hour or so and finally over night at room temperature. It was then filtered, washed, dissolved in an excess of standard alkali and determined volumetrically by titration with standard acid.

Potassium was determined gravimetrically by evaporating an aliquot directly with  $\text{H}_2\text{PtCl}_6$ , filtering and washing the precipitate with acid alcohol (1 part concentrated  $\text{HCl}$  to 10 parts 95 per cent  $\text{C}_2\text{H}_5\text{OH}$ ) and  $\text{NH}_4\text{Cl}$  solution to remove impurities and finally with 80 per cent alcohol.

Calcium was determined by precipitating it as  $\text{CaC}_2\text{O}_4$  with hot  $(\text{NH}_4)_2\text{C}_2\text{O}_4$  solution added to the boiling solution previously slightly acidified with  $\text{HCl}$ . The whole was boiled for 2-3 minutes, and allowed to stand over night. The precipitate was then filtered, washed, heated with dilute  $\text{H}_2\text{SO}_4$ , and the calcium determined volumetrically with standard  $\text{KMnO}_4$  solution.

Silicon was determined in the usual manner by treating the weighed residue of dehydrated silica obtained in the method with hydrofluoric acid and a little sulfuric acid to eliminate impurities.

#### *Short fifth-normal nitric acid method*

Two procedures were used. In one, the soil was digested 5 minutes, shaking every minute, before pouring upon the filter. In the other, the soil was put upon the filter and the acid poured through it. The latter is designated in the tables as "Digestion = 0 minutes." In other respects, both were similar to the 5-hour extraction. Except where noted, 120 gm. of air-dry surface soil was used and where comparisons have been made the digestions were carried on together on equal quantities of soil. Some of the results in the tables are averages. Most, however, are individual determinations. As no particular difficulty was experienced in obtaining fairly close duplicates, it was not thought necessary to make them in every case.



*Effect of time on the acid digestion*

Some experiments have been made in which the soil was digested in 0.2 N  $\text{HNO}_3$  for different periods. The results are given in table 1.

## COMPARISON OF VIRGIN AND CULTIVATED SOILS

Since nearly as much phosphorus, potassium and calcium was found in solution in the short as in the longer acid extraction, the effect of similar

TABLE 1

*Plant-food elements extracted from air-dried soils when digested in 0.2 N  $\text{HNO}_3$  for different periods of time*

SOIL NUMBER	PHOSPHORUS			POTASSIUM			CALCIUM		
	0 min. utes*	5 minutes	5 hours	0 minutes	5 minutes	5 hours	0 minutes	5 minutes	5 hours
	p. p. m.	p. p. m.	p. p. m.	p. p. m.	p. p. m.	p. p. m.	p. p. m.	p. p. m.	p. p. m.
43506	8		7	61		114	1000		1100
43526	10		13	51		63	540		410
50117	19		16	52		63	240		460
56527†	10	12	10	105	122	138	1680	1880	1870
56528§	25		22	79		98	790		840
56529†	9	12	8	88	99	118	510	490	510
56530†	12	14	14	113	132	154	970	1030	1080
56532‡	10	10	11	51	78	93	960	1050	1140
56583	34		43	104		137	470		540
56584	16	15	16	35	42	43	560	520	580
56585	16	30	18	56	84	95	790	990	1020
56586	17	18	12	105	153	183	460	590	630
56587	14	12	13	80	119	124	480	580	620
56588	21	25	19	88	100	114	890	910	1020
56592	13	10	9	96	117	135	750	790	800
56652	9		5	115		121	740		670
56654	11		5	37		44	540		
Average†.....	14	16	13	82	105	120	805	883	927
Relative solubility.....	108	123	100	77	88	100	87	95	100

\* See p. 385.

† Only those samples are included in which digestions were made for the three periods.

‡ 100 gm. of soil taken for the digestion.

§ 110 gm. of soil taken for the digestion.

digestions on the soil silicates was determined. Several comparisons have also been made of virgin with the corresponding cultivated soil. The results are given in table 2.

## EFFECT OF TOBACCO CULTIVATION ON SOME SOIL CONSTITUENTS

It is generally believed in this locality that tobacco rapidly impoverishes the soil, possibly due to the fact that a comparatively large yield is obtained

on good land and this from a plant with shallow roots which necessarily derives most of its sustenance from the surface layer. Moreover it has a very high ash and withdraws relatively large amounts of potassium, nitrogen and calcium from the soil. For this reason it is generally grown on virgin or old sod land and after two or three crops have been removed the land is put in clover or grass to restore its fertility. Some digestions were made of such soils and also of the corresponding virgin samples. Most of the soils worked upon contain considerable calcium phosphate which is naturally unevenly distributed. As this material is gradually soluble in 0.2 N  $\text{HNO}_3$ , this probably accounts for some irregularities and high figures obtained for phosphorus and calcium, especially in the 5-hour extraction. The results are given in table 3.

#### EFFECT OF AIR DRYING THE SOIL AND THE PRESENCE OF LIMESTONE IN SAME ON THE 0.2 N $\text{HNO}_3$ DIGESTION

Some experiments have been made in determining what effect digesting the moist soil in 0.2 N  $\text{HNO}_3$  without preliminary air-drying would have on the results obtained as compared with the air-dried sample. Experiments have also been made on testing the effect produced by limestone on the solubility of plant-food constituents when it has been in contact with the moist soil some time previous to the acid digestion. In all of these experiments, the moisture content of the soil was maintained at 20 per cent for several weeks previous to the acid extraction. Moist soil equivalent to the usual quantity of the moisture free sample employed was used for the digestion. In the moist soils which contained limestone stronger acid was employed as explained under "Methods." The water present in the moist soil was also considered in the preparation of the solvent so that the latter would not be diluted in the extraction. The limestone used was practically pure  $\text{CaCO}_3$  and was 100-mesh fine. It was thoroughly mixed with the soil at the rate of 4000 parts per million of soil. The required amount of water was then added to maintain the above moisture content. The results are given in table 4 together with those obtained on the air dried samples recorded in tables 1 and 2.

#### EFFECT OF VARIABLE AMOUNTS OF SOIL IN THE ACID DIGESTION

Some of the foregoing experiments indicate that adsorption exerts a considerable influence on the results obtained. If this is true, then it may be influenced by the character and possibly to some extent by the quantity of soil used as well as by the duration of the extraction. In order to determine if quantity was a factor, experiments have been made in digesting different amounts of the same soil in their proportionate volumes of acid. The results are given in table 5.

TABLE 2

*Amounts of plant-food elements extracted from air-dried soils when digested in 0.2 N HNO<sub>3</sub> for different periods of time*

SOIL NUMBER	CHARACTER	PHOSPHORUS		POTASSIUM		CALCIUM		SILICON	
		5 minutes	5 hours	5 minutes	5 hours	5 minutes	5 hours	5 minutes	5 hours
		p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.
2305	Cult. 9 years.	153	145	138	157	3640	3730	63	237
2306	Cult. 33 years.	25	23	101	117	2580	2570	53	191
9768	Virgin	62	62	206	219	6050	6500	90	279
9771	Cult.	15	19	80	92	920	940	22	93
14411	Cult.	32	36	153	170	3220	3240	33	153
14412	Virgin	146	191	451	496	4420	4860	58	242
17483	Cult.	126	158	144	208	2290	2680	32	155
17485	Virgin	113	122	206	188	1780	1930	33	145
25002	Cult.	4	8	124	120	790	1020	36	106
25004	Virgin	10	15	200	222	1290	1350	34	88
25662	Cult.	13	9	132	150	710	760	22	84
25663	Virgin	21	15	102	132	380	400	24	82
25796	Cult.	27	31	92	105	1690	1770	46	177
25797	Virgin	55	74	202	227	1920	2070	62	220
36263	Virgin	9	9	77	84	310	280	14	53
36538	Virgin	27	26	205	230	3400	3560	70	118
36539	Cult.	17	11	185	207	1630	1700	61	99
36694	Cult.	28	43	64	74	790	860	18	85
36696	Virgin	42	51	149	162	1330	1410	33	139
36792	Virgin	27	28	106	118	880	870	32	130
36796	Cult.	23	17	87	93	500	510	24	83
56447	Cult.	8	10	156	174	650	690	18	51
54449	Virgin	20	21	250	256	3030	3140	47	108
56489	Virgin†	4	6	124	137	1020	1080	25	98
56490	Cult.‡	4	6	106	117	820	840	41	116
56491	Virgin‡	3	6	126	140	650	720	28	79
56492	Cult.‡	4	6	61	72	1090	1130	36	101
56493	Virgin	11	14	225	256	2830	2710	41	137
56495	Cult.	5	8	68	72	820	850	16	84
56497	Cult.	14	15	84	99	730	750	24	84
56499	Virgin	20	23	226	244	2090	2110	36	130
56501	Cult.	84	109	82	86	3980	3770	56	382
56503	Virgin	104	125	95	104	4600	4710	68	383
56525	Cult.	7	7	86	63	300	340	12	26
56526	Cult.	19	22	153	174	1140	1230	49	220
56531†	Cult.	12	13	97	69	260	280	38	73
56534	Cult.	4396	4816	70	97	13810	16020		
56549	Cult.	19	28	68	79	830	780	29	107
56699	Cult.	6	11	91	103	2000	2050	86	277
56700	Cult.	17	21	118	135	1010	1000	36	160

TABLE 2—Continued

SOIL NUMBER	CHARACTER	PHOSPHORUS		POTASSIUM		CALCIUM		SILICON	
		5 minutes	5 hours	5 minutes	5 hours	5 minutes	5 hours	5 minutes	5 hours
		p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.
56701	Cult.	6	6	107	119	1470	1290	141	182
56702	Cult.	11	14	48	53	420	380	26	127
56727	Cult.	23	28	120	142	2380	2500	86	392
56728	Cult.	79	83	148	178	3610	3770	189	625
56733	Cult.	51	61	82	87	1090	1160		
56740	Cult.	5	6	58	68	1530	1720	70	213
56741	Cult.	6	7	87	96	660	750	26	139
56742	Cult.	8	10	104	125	1280	1350	29	153
61036	Cult.	5	9	54	58	720	800	23	127
61037	Cult.	5	9	58	67	890	930	24	100
61122	Cult.	5	12	91	105	1060	1100	23	134
61123	Cult.	6	10	60	68	580	600	13	93
61244	Cult.	4	9	108	123	690	660	32	89
61246	Virgin	5	8	106	116	250	230	10	44
61253	Cult.	5	8	128	140	350	350	19	68
61254	Cult.‡	4	7	108	113	310	320	55	172
61255	Virgin	6	9	87	92	100	200	18	43
61256	Virgin§	5	6	97	100	170	180	34	123
61265	Cult.	4	7	63	69	790	820	20	105
61266	Cult.‡	7	9	66	70	420	420	38	124
61267	Virgin	3	8	63	66	310	310	22	91
61268	Virgin§	2	7	69	66	130	160	38	146
Average*		26	30	121	133	1442	1501	88	316
Relative solubility		87	100	91	100	96	100	28	100

\* Only those samples are included in which the silicon was also determined,

† 10 gm. of soil taken for the digestion.

‡ Sample of the first 18 inches.

§ Subsoil of the preceding soil at depth of 6–18 inches.

## DISCUSSION OF RESULTS AND CONCLUSIONS

There have been 92 soils used in this investigation, representing the different soil areas found in this state. The results show that there are 30 samples in which the percentage of phosphorus, 5 in which the percentage of potassium and 16 in which the percentage of calcium found by the short digestion equals or exceeds the percentages of these elements obtained by the longer extraction. The silicon obtained, however, by the short digestion is less in every sample. If a direct comparison be made of the figures obtained for each element where it was determined in the same sample, both by the 5 minute and 5 hour digestion, it will be found that there are 73 soils in which the silicon was determined and 85 soils in which only phosphorus, potassium and calcium were estimated. Taking the averages represented by these

samples, it will be found that 91 per cent of the phosphorus, 89 per cent of the potassium, 94 per cent of the calcium and only 28 per cent of the silicon are obtained in the short digestion as compared with the regular procedure. If the averages of all soils used are considered, the results are still better, namely, 97 per cent, 92 per cent, 99 per cent and 28 per cent, respectively, for the above elements.

The experiments indicate that adsorption in some soils is a factor which may appreciably affect the results obtained for certain plant-food constituents in the 0.2 *N*  $\text{HNO}_3$  digestion as ordinarily employed. With such soils, there-

TABLE 3  
*Effect of tobacco cultivation on the amounts of plant food elements extracted from air-dried soils when digested in 0.2 N  $\text{HNO}_3$  for different periods of time*

SOIL NUMBER	CHARACTER	PHOSPHOROUS		POTASSIUM		CALCIUM		SILICON	
		5 minutes	5 hours	5 minutes	5 hours	5 minutes	5 hours	5 minutes	5 hours
		<i>p. p. m.</i>	<i>p. p. m.</i>	<i>p. p. m.</i>	<i>p. p. m.</i>	<i>p. p. m.</i>	<i>p. p. m.</i>	<i>p. p. m.</i>	<i>p. p. m.</i>
56747	Virgin	954	780	180	222	27460	29370	181	394
56748	Tobacco, 2 years.	246	234	142	169	5840	6200	124	350
56959	Cult. good crop*	24	24	73	90	1910	2150	31	120
56960	Cult. poor crop	24	18	47	54	1780	1850	34	132
61192	Cult. good crop†	14	25	107	119	950	970	37	145
61193	Cult. poor crop‡	8	8	24	32	720	770	28	117
61194	Virgin	129	148	225	258	2600	2760	36	170
61195	Tobacco, 2 years	112	165	247	288	2480	2530	33	159
61196	Tobacco, 3 years	166	239	106	130	1990	2480	23	142
61197	Virgin	296	370	264	303	3490	3900	44	214
61198	Tobacco 2 years	278	378	211	254	3530	3740	56	223
61199	Virgin	429	464	271	306	3950	4250	48	242
61200	Tobacco, 2 years	535	626	187	217	5340	6030	75	302
Average.....		247	268	160	188	4772	5154	58	209
Relative solubility.....		92	100	85	100	93	100	28	100

\* This land was not in tobacco. The good crop was on land in cultivation 1 year while soil no. 56960, with the same soil characteristics, had been cultivated several years, part of the time in tobacco.

† From a field of corn where the growth was good.

‡ From the same field where the growth was poor.

fore, the result usually reported for the prescribed 5-hour period represents the quantity dissolved by the acid during this time less the amount subsequently withdrawn from solution by the soil during the same time due to adsorption. Assuming that the latter is not an instantaneous process, the less time that the soil is in contact with the acid would be advantageous in partly overcoming this effect. The short digestion is, therefore preferable on this account inasmuch as the averages obtained in this work show that it gives about as good results and moreover shortens the regular procedure.

TABLE 4  
Effect of air-drying and addition of limestone on the amounts of plant food elements extracted from moisture-free soils when digested in 0.2 N HNO<sub>3</sub>

NUMBER	TREATMENT	PHOSPHORUS (DIGESTION = 0 MINUTES§)	POTASSIUM (DIGESTION = 0 MINUTES§)	CALCIUM (DIGESTION = 0 MINUTES§)
		p.p.m.	p.p.m.	p.p.m.
56584	Moist.....	19	40	570
	Moist, limed.....	18	27	2110
	Air-dried.....	16	36	570
56585	Moist.....	31	83	1030
	Moist, limed.....	32	70	2520
	Air-dried.....	16	57	800
56586	Moist.....	21	148	580
	Moist*.....	16	153	630
	Moist, limed.....	22	127	1880
	Moist, limed*.....	18	145	1990
	Air-dried.....	17	107	470
56587	Moist.....	15	116	660
	Moist, limed.....	18	111	2210
	Air-dried.....	14	81	490
56588	Moist.....	23	102	1010
	Moist, limed.....	26	91	2460
	Air-dried.....	21	89	900
56592	Moist.....	13	134	820
	Moist*.....	11	112	800
	Moist, limed.....	15	134	2400
	Moist, limed*.....	12	108	2170
	Air-dried.....	13	97	760
56652	Moist.....	9	98	760
	Moist, limed.....	13	116	—
	Air-dried.....	9	117	750
56654	Moist.....	9	25	510
	Moist, limed.....	10	11	—
	Air-dried.....	11	38	550
56699	Moist, limed*.....	6	86	2020
	Air-dried, limed†.....	6	92	2030
56700	Moist*.....	16	116	1000
	Air-dried†.....	17	120	1030
56701	Moist*.....	7	100	1340
	Air-dried†.....	6	109	1490
56702	Moist*.....	11	48	420
	Air-dried†.....	11	49	430
56740	Air-dried†.....	5	—	1550
	Moist, limed†.....	7	—	1640
56741	Air-dried†.....	6	—	670
	Moist†.....	6	—	760
56742	Air-dried†.....	8	—	1300
	Moist†.....	8	—	1420

\* Digested for 5 minutes.

† Digested soil for 5 minutes.

‡ Digested amount equivalent to 50 gm. air-dried soil in 500 cc. of 0.2 N HNO<sub>3</sub>. This was one-half of usual quantities of soil and acid employed.

§ See page 385.

TABLE 5  
Effect of variable amounts of soil on the quantities of plant-food elements extracted when digested  
in 0.2 N HNO<sub>3</sub>

SOIL NUMBER	AMOUNT OF SOIL USED <sup>§</sup>	PHOSPHORUS (DIGESTION = 5 MINUTES)	POTASSIUM (DIGESTION = 5 HOURS)	CALCIUM (DIGESTION = 5 MINUTES)
	gm.	p.p.m.	p.p.m.	p.p.m.
43506	120	....	114	
	50	....	89	
43526	120	....	63	
	50	....	74	
50117	120	....	63	
	50	....	72	
56525	120	7	63	300
	50	10	95	350
56526	120	19	174	1140
	50	40	167	1430
56527	100	12	138	1880
	50	14	164	1960
56528	110	25 <sup>b</sup>	98	790*
	50	33 <sup>b</sup>	101	980*
56529	100	12	118	490
	50	12	114	620
56530	100	14	...	1030
	50	9	...	1100
56531	110	12	...	260
	50	15	...	360
56532	100	10	93	1050
	50	5	64	1110
56584	120	....	43	
	50	....	46	
56586	120	....	183	
	50	....	167	
56592	120	....	135	
	50	....	169	
56700	120	....	135	
	50	....	132	
56702	120	....	53	
	50	....	59	
56740	120	5	...	1530
	50	7	...	1640
56741	120	6	...	660
	50	6	...	760
56742	120	8	...	1280
	50	8	...	1420
56747	120	780†	222	29370†
	50	744†	216	29320†
56748	120	234†	169	6200†
	50	196†	164	6110†

<sup>§</sup> The relative proportions used were 1 gm. soil to 10 cc. of 0.2 N HNO<sub>3</sub>

\* Digestion = 0 minutes, see p. 385.

† Digestion = 5 hours.

It will probably be conceded that the solvent used in any method of this character should have very little action on the soil silicates. The fact that they are not attacked to the same extent in the short digestion as shown for the silicon in tables 2 and 3 while the bulk of the other elements are obtained makes this procedure look more favorable for the purpose intended.

That the weak acid digestion as employed here does have some value for purposes of comparison at least for indicating possible deficiencies of plant food elements in soils is shown in the same tables. In making comparisons by this method, however, it is essential that the extractions be carried on together and on the same quantity of soil. This latter point is demonstrated in table 5.

The addition of calcium carbonate to the soil as shown in table 4 increases the amount of phosphorus and decreases the amount of potassium found in the 0.2 *N*  $\text{HNO}_3$  extract of most of these samples. On the other hand, failure to dry the sample for the acid digestion appears to exert a variable influence on the amount of the above elements found in solution as compared with a similar digestion of the air dried soil. For instance, in table 4, among the soils worked upon, there are some where the amounts of both of these elements are increased when the moist soil was used but on the contrary there are about as many which show either no effect or a loss. For this reason it is difficult to draw general conclusions regarding this factor as this seems to depend on the character of the soil.

Notwithstanding that it is an empirical procedure, the writer believes that the weak acid digestion, especially of short duration as employed in this investigation is valuable for indicating possible deficiencies of plant food elements in soils, provided it is used for purposes of comparison or in connection with a soil survey.

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## VARIABILITY OF NITRATES AND TOTAL NITROGEN IN SOILS<sup>1</sup>

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The reliability of chemical determinations on soils, in their application to large or small areas of land of apparent uniformity, has been given much consideration by investigators recently. It is well recognized that a large plot of land, even if the soil were apparently uniform in appearance, would require a large number of samples, composited and analyzed separately in order to give a reliable index for the whole area. Waynick (8) and Waynick and Sharp (9) have called attention to the great variability of nitrates and also the extreme variation in nitrogen and carbon content in field samples of soil.

In the plot work at the New Jersey Experiment Station, there has been a question in the mind of the writer as to how many samples should be taken for chemical work in order to secure results representative of the entire plot. The plots cover a relatively small area, ( $\frac{1}{100}$  of an acre), and it would seem that a few samples, carefully taken and covering the plot should, when composited, fairly represent the area. An attempt has been made to study this problem by determining the nitrates and total nitrogen content of a large number of samples from three plots which have received different fertilizer treatment for the past 15 years. In studies on the same plots, Waksman (7) has recently shown the importance which the numbers of microorganisms play in indicating the fertility of a soil. Probably in the majority of cases nitrates would tend to be present in largest amounts in the more fertile soils. However, Blair and Prince (2) have shown that nitrates may be formed in considerable quantities in a soil that is so acid as to practically inhibit growth of ordinary farm crops. An opportunity was offered in the present work to compare soils under different fertilizer treatment with reference to their nitrate content and microbial flora.

### METHODS

The plots selected for this work belong to the series at the New Jersey Agricultural Experiment Station, which is being used in a study of the availability of nitrogenous fertilizers. The plots are one-twentieth of an acre in size and were laid out in 1908. Since then they have been under the same system of fertilization and cropping. The soil is a Sassafraus loam. The

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<sup>2</sup> The writer is indebted to Dr. S. A. Waksman for suggesting the problem and for valuable suggestions during the progress of the work.

rotation is a general farm crop rotation—corn, oats, wheat, and 2 years of timothy. The details of the experiment together with the chemical analyses of the soil and crops are reported in earlier papers by Lipman and Blair (3, 4). The particular plots chosen for this work were 5A, 7A, and 9A. These plots have received no lime during the period. The fertilizer treatment on the acre basis for these plots has been as follows: plot 5A, 640 pounds acid phosphate, 320 pounds potassium chloride and 16 tons of cow manure annually; plot 7A, nothing; plot 9A, 640 pounds acid phosphate, 320 pounds potassium chloride and 320 pounds sodium nitrate annually. The total yield of dry matter (hay, straw, grain) for the past 15 years has been 70,191 pounds for plot 5A; 15,774 pounds for plot 7A; and 57,848 pounds for plot 9A.

Twenty-five samples were taken from each plot during August, 1922 and distributed as shown in figure 1. The plots are 65.87 feet by 33.36 feet wide so that each sample was about 8–10 feet from those adjacent. In taking samples a tube cutting a core about one inch in diameter was used. Three such borings to the depth of 6½ inches were taken within a radius of 5–6 inches for each sample. The soil was quite moist, although it had not rained for about one week. The samples were brought to the laboratory in covered pint jars. The following day they were passed through a 5-mm. sieve and thoroughly mixed. There was only a very small amount of detritus (10–20 gm). In order to study not only the variation between the samples, but also to learn if there was a correlation between nitrates and the nitrifying capacity of the soil, all the samples were tested for their nitrifying power. One hundred grams were tested for their nitrifying power. One hundred grams of the soil from each of the samples was weighed into tumblers. Thirty milligrams of nitrogen in the form of ammonium sulfate was used as a source of nitrogen. It is known that  $(\text{NH}_4)_2\text{SO}_4$  tends to make a soil acid, and consequently the microbial flora would be affected and nitrification processes interfered with. In order to prevent, as nearly as possible, any change in the original acidity of the soils, 0.2 gm. calcium carbonate was added to each tumbler to neutralize the acids which would be formed from the addition of ammonium sulfate. The soil in the tumblers was stirred thoroughly and allowed to incubate at 28°C. for 28 days, keeping it at an optimum moisture content. Moisture determinations were run on several of the original samples from each plot. Plot 5A averaged 11.5 per cent water; plot 7A, 9.5 per cent and plot 9A, 12.5 per cent water.

The remainder of the soil from the original samples was air-dried and passed through a 2-mm. sieve. Nitrate determinations were made on each sample by the phenoldisulfonic acid method (5). At the expiration of 28 days, the soils in the nitrification experiments were spread out and allowed to dry, then passed through a 2-mm. sieve and the nitrates determined as above. The results for the nitrate determinations on the untreated soils are given in table 1 and for the nitrification studies, in table 2. A composite sample was made by taking 25 gm. from each individual sample. This was mixed thoroughly and ten determinations for nitrates made on each composite. The results are given in table 3.

The mean, coefficient of variability, and probable error of the mean have been calculated for each plot. The methods of calculation were those employed by Waynick (8) and also by Waksman (6). The standard deviation, which is represented by  $\delta$  is found by squaring the deviation from the mean, taking the sum of the squares thus found, dividing this figure by the total number of determinations and taking the square root of the quotient. The coefficient of variability  $C. V.$  is simply the percentage ratio of the standard deviation to the mean. The probable error of the mean  $Em$  is given by the formula:

$$Em = \frac{0.6745 \times \delta}{\sqrt{N}}$$

where  $N$  stands for the number of determinations.

#### DISCUSSION OF TABLES 1, 2, AND 3

The most striking observation to be noticed from table 1 is the great variation in the nitrate content existing between the various samples within each plot. The coefficient of variability for plot 5A is 34.7 per cent, which

means that approximately two-thirds of the determinations may be expected to lie within that range on either side of the mean. That is, the range within which two-thirds of the determinations may be expected to fall is 0.31-0.67 milligrams in this case. The extreme range would of course be much greater.

TABLE 1  
*Nitrate in soil as sampled*

SAMPLE NUMBER	PLOT 5A		PLOT 7A		PLOT 9A	
	Nitrate nitrogen	Deviation from mean ±	Nitrate nitrogen	Deviation from mean ±	Nitrate nitrogen	Deviation from mean ±
	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
1	0.41	0.08	0.16	0.01	0.32	0.00
2	0.56	0.07	0.13	0.04	0.36	0.04
3	0.51	0.02	0.15	0.02	0.35	0.03
4	0.62	0.13	0.17	0.00	0.43	0.11
5	0.51	0.02	0.13	0.04	0.42	0.10
6	0.67	0.18	0.14	0.03	0.43	0.11
7	0.53	0.04	0.15	0.02	0.34	0.02
8	0.54	0.05	0.17	0.00	0.45	0.13
9	0.26	0.23	0.15	0.02	0.34	0.02
10	0.26	0.23	0.17	0.00	0.44	0.12
11	0.29	0.20	0.25	0.08	0.42	0.10
12	0.19	0.30	0.19	0.02	0.32	0.00
13	0.64	0.15	0.15	0.02	0.34	0.02
14	0.61	0.12	0.14	0.03	0.34	0.02
15	0.66	0.17	0.21	0.04	0.19	0.13
16	0.58	0.09	0.16	0.01	0.34	0.02
17	0.28	0.21	0.15	0.02	0.19	0.13
18	0.58	0.09	0.17	0.00	0.24	0.08
19	0.46	0.03	0.17	0.00	0.25	0.07
20	0.51	0.02	0.16	0.01	0.24	0.08
21	0.66	0.17	0.19	0.02	0.16	0.16
22	0.96	0.47	0.22	0.05	0.45	0.13
23	0.30	0.19	0.19	0.02	0.18	0.14
24	0.27	0.22	0.25	0.08	0.34	0.02
25	0.30	0.19	0.17	0.00	0.29	0.03
Mean....	0.49 mgm.	0.15 mgm.	0.17 mgm.	0.02 mgm.	0.32 mgm.	0.07 mgm.
$\sigma$ .....	0.18		0.03		0.09	
C.V.....	34.7%		17.7%		28.1%	
Em.....	0.024 mgm.		0.004 mgm.		0.012 mgm.	
	or		or		or	
	4.95%		2.38%		3.80%	

The coefficient of variability for plot 7A is 17.7 per cent and for 9A, it is 28 per cent. Since plot 5A has been highly manured, it would be expected to show the greatest variation in nitrate content as, in reality, it does; while plot 7A which has received no fertilizer treatment, is the least variable. The variation is also great in 9A where sodium nitrate had been applied. The probable errors of the mean in each case vary in the same proportion.

Table 2 which records data on the nitrification studies also shows much variation among the individual samples, but the coefficient of variability and probable errors of the mean on plot 5A and 9A are much reduced, while the variation between samples on 7A is increased immensely. This would indicate

TABLE 2  
*Nitrate produced in 100 gm. of soil from ammonium sulfate in 28 days*

SAMPLE NUMBER	PLOT 5A		PLOT 7A		PLOT 9A	
	Nitrate nitrogen	Deviation from mean $\pm$	Nitrate nitrogen	Deviation from mean $\pm$	Nitrate nitrogen	Deviation from mean $\pm$
	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
1	24.0	0.2	2.4	1.8	11.6	5.8
2	26.4	2.6	3.2	1.0	19.2	1.8
3	27.2	3.4	3.4	0.8	18.8	1.4
4	27.2	3.4	2.5	1.7	16.0	1.4
5	28.0	4.2	2.2	2.0	13.6	3.8
6	28.0	4.2	3.0	1.2	18.0	1.4
7	27.2	3.4	2.6	1.6	17.6	0.2
8	25.6	1.8	4.5	0.3	20.0	2.6
9	24.8	1.0	2.2	2.0	19.2	1.8
10	25.6	1.8	3.4	0.8	19.6	2.2
11	25.6	1.8	3.4	0.8	20.0	2.6
12	25.6	1.8	3.6	0.6	22.0	4.6
13	23.2	0.6	4.0	0.2	22.4	5.0
14	24.8	1.0	2.8	1.4	14.8	2.6
15	24.8	1.0	4.3	0.1	16.8	0.6
16	24.0	0.2	3.7	0.5	16.0	1.4
17	24.0	0.2	4.2	0.0	19.6	2.2
18	23.2	0.6	5.0	0.8	19.2	1.8
19	19.2	4.6	5.4	1.2	17.6	0.2
20	21.8	2.0	3.6	0.6	12.0	5.4
21	20.8	3.0	5.6	1.4	13.2	4.2
22	21.3	2.5	5.7	1.5	17.6	0.2
23	18.6	5.2	8.0	3.8	15.6	1.8
24	17.0	6.8	8.4	4.2	16.0	1.4
25	17.0	6.8	8.2	4.0	19.2	1.8
Mean . . .	23.8 mgm.	2.6 mgm.	4.2 mgm.	1.4 mgm.	17.4 mgm.	2.3 mgm.
$\sigma$ . . . . .	3.19		1.77		2.82	
C.V. . . . .	13.4%		42.1%		16.2%	
Em. . . . .	0.431 mgm.		0.239 mgm.		0.380 mgm.	
	or		or		or	
	1.81%		5.68%		2.18%	

that certain sections of plot 7A are capable of facilitating nitrification processes more rapidly than others. The upper half of the plot (samples 15-25) gave by far the highest yields of nitrates on this plot. It will also be noted in table 1 that samples 13, 14, 15, and 16 which lie in the center of the plot, yielded very high nitrates for plot 5A. These samples lie in a straight line

across the plot and no doubt indicate that there was an accumulation of manure and microorganisms in this section. This may have been due to ploughing which was done in this direction.

In table 3, the nitrates are given for the composite sample made from the 25 individual samples. In all cases the mean runs considerably lower than the mean for the 25 individual samples. It is difficult to explain this discrepancy, unless it is due to the fact that the determinations on the composite samples were not made until a month later. However, as all the samples

TABLE 3  
*Nitrate in soil-composite of twenty-five samples*

SAMPLE NUMBER	PLOT 5A		PLOT 7A		PLOT 9A	
	Nitrate nitrogen	Deviation from mean ±	Nitrate nitrogen	Deviation from mean ±	Nitrate nitrogen	Deviation from mean ±
	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
1	0.34	0.03	0.12	0.01	0.25	0.01
2	0.36	0.01	0.12	0.01	0.24	0.00
3	0.37	0.00	0.11	0.00	0.21	0.03
4	0.37	0.00	0.12	0.01	0.25	0.01
5	0.37	0.00	0.10	0.01	0.24	0.00
6	0.38	0.01	0.10	0.01	0.22	0.02
7	0.40	0.03	0.11	0.00	0.25	0.01
8	0.35	0.02	0.10	0.01	0.26	0.02
9	0.35	0.02	0.12	0.01	0.23	0.01
10	0.36	0.01	0.12	0.01	0.23	0.01
Mean....	0.365 mgm.	0.013 mgm.	0.11 mgm.	0.008 mgm.	0.24 mgm.	0.012 mgm.
σ.....	0.017		0.0089		0.0148	
C.V.....	4.66%		8.09%		6.17%	
Em.....	0.0036 mgm.		0.0019 mgm.		0.0032 mgm.	
	or		or		or	
	1.0%		1.72%		1.31%	

were dried at the same time, this should make no difference. The coefficient of variability and probable error of the mean on the composite samples are relatively low and indicate that the number of determinations on a well composed sample need be but few.

In order to study the data further, table 4 has been prepared. Here the mean of the ten lowest and ten highest nitrate determinations on the original soil is compared with the corresponding values for the same samples incubated with ammonium sulfate. The lowest and highest individual values and the mean of the twenty-five samples from each plot are also recorded. From the table it is clear that the original samples which are lowest in nitrate nitrogen also show the lowest nitrifying power while the original samples which are highest in nitrate nitrogen show a tendency toward greater nitrifying ability.

## TOTAL NITROGEN DETERMINATIONS

The second part of the work consisted in studying the variability of the total nitrogen content of the individual samples from each plot. The soils were passed through a 1-mm. sieve and the total nitrogen determined in duplicate on each individual sample by the ordinary Kjeldahl method (1). Ten determinations were also run on the composite sample from each plot made from the twenty-five individual samples. The data are given in tables 5 and 6. The coefficient of variability on all the plots is comparatively low for this determination, being not much over 5 per cent and the probable error of the mean is in all cases less than 1 per cent. The mean values are, for the three plots, decidedly different and correspond to their relative productivity.

TABLE 4

*Comparison of the mean values for the ten lowest and highest soil nitrates and for the corresponding samples treated with  $(\text{NH}_4)_2\text{SO}_4$*

	PLOT 5A		PLOT 7A		PLOT 9A	
	Nitrate nitrogen in untreated soil	Nitrate nitrogen in $(\text{NH}_4)_2\text{SO}_4$ -treated soil	Nitrate nitrogen in untreated soil	Nitrate nitrogen in $(\text{NH}_4)_2\text{SO}_4$ -treated soil	Nitrate nitrogen in untreated soil	Nitrate nitrogen in $(\text{NH}_4)_2\text{SO}_4$ -treated soil
	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
Mean of 25 samples.....	0.49	23.8	0.17	4.2	0.32	17.4
Mean of 10 lowest nitrate samples.	0.30	22.1	0.15	3.0	0.25	16.2
Mean of 10 highest nitrate samples.	0.65	26.4	0.21	5.0	0.40	18.0
Lowest individual value.....	0.19	17.0	0.13	2.2	0.16	11.6
Highest individual value.....	0.96	28.0	0.25	8.4	0.45	22.4

The chief point to note in the discussion of this phase of the work is that the variability of total nitrogen content of soils is not nearly as great as in the case of the nitrates. One reason for this is the fact that the method of determining total nitrogen is not nearly as sensitive as the method for the determination of nitrates and the field errors of sampling are, consequently minimized. In the ordinary methods for the determinations of phosphorus and potassium, the same general tendency would probably hold true. That is, these methods are not sufficiently delicate to cause appreciable variability among a large number of samples taken from a small area, such as occurs, in the determination of nitrates. It would seem, therefore, quite unnecessary in determinations of this sort to have such a large number of samples. It is interesting to note from table 6 how close the mean values are for the composite samples as compared with the mean values for the twenty-five individual samples. In each case the difference is only one in the third place. The table also shows that it is unnecessary to run more than three determinations for nitrogen on a sample well composited.

It has been the customary practice in taking samples for chemical work from the experimental plots at this station to make a composite of about 9-12

individual samples covering the plot. In order to get an approximate idea of how truly representative such a sample would be, the mean of nine such samples covering the plot has been taken and compared with the mean of the total twenty-five samples. The nine chosen were samples 1, 4, 7, 9, 12, 17,

TABLE 5  
*Total nitrogen in individual soils samples*

SAMPLE NUMBER	PLOT 5A		PLOT 7A		PLOT 9A	
	Nitrogen content	Deviation from mean ±	Nitrogen content	Deviation from mean ±	Nitrogen content	Deviation from mean ±
	gm. per 100	gm. per 100	gm. per 100	gm. per 100	gm. per 100	gm. per 100
1	0.143	0.005	0.070	0.006	0.093	0.011
2	0.139	0.001	0.071	0.005	0.099	0.005
3	0.136	0.002	0.074	0.002	0.108	0.004
4	0.126	0.012	0.076	0.000	0.103	0.001
5	0.132	0.006	0.072	0.004	0.094	0.010
6	0.138	0.000	0.073	0.003	0.103	0.001
7	0.138	0.000	0.073	0.003	0.103	0.004
8	0.130	0.008	0.080	0.004	0.104	0.000
9	0.135	0.003	0.075	0.001	0.107	0.003
10	0.147	0.009	0.072	0.004	0.101	0.003
11	0.137	0.001	0.082	0.006	0.115	0.011
12	0.135	0.003	0.084	0.008	0.113	0.009
13	0.148	0.010	0.076	0.000	0.109	0.004
14	0.154	0.016	0.070	0.006	0.102	0.002
15	0.136	0.002	0.071	0.005	0.110	0.006
16	0.130	0.008	0.080	0.004	0.110	0.006
17	0.138	0.000	0.082	0.006	0.098	0.006
18	0.142	0.004	0.072	0.004	0.101	0.003
19	0.135	0.003	0.076	0.000	0.103	0.001
20	0.139	0.001	0.080	0.004	0.107	0.003
21	0.143	0.005	0.081	0.005	0.096	0.008
22	0.159	0.021	0.075	0.001	0.096	0.008
23	0.138	0.000	0.076	0.000	0.102	0.002
24	0.142	0.004	0.080	0.004	0.106	0.002
25	0.143	0.005	0.080	0.004	0.112	0.008
Mean....	0.138 gm.	0.0052 gm.	0.076 gm.	0.0036 gm.	0.104 gm.	0.0048 gm.
σ.....	0.0073		0.0042		0.0058	
C.V.....	5.29%		5.40%		5.57%	
Em.....	0.00098 gm.		0.00057 gm.		0.00078 gm.	
	or		or		or	
	0.713%		0.745%		0.75%	

20, 23, and 25 as shown on figure 1. These figures represent points where it has been customary to take samples. From table 7, it will be seen that in the case of nitrates there are some differences between the mean of the nine and the twenty-five samples, but for most practical purposes these differences are unimportant. For instance, the mean of the nine samples for nitrates brings



TABLE 6  
Total nitrogen in soil-composite of twenty-five samples

SAMPLE NUMBER	PLOT 5A		PLOT 7A		PLOT 9A	
	Nitrogen content	Deviation from mean $\pm$	Nitrogen content	Deviation from mean $\pm$	Nitrogen content	Deviation from mean $\pm$
	gm. per 100	gm. per 100	gm. per 100	gm. per 100	gm. per 100	gm. per 100
1	0.136	0.001	0.076	0.001	0.103	0.000
2	0.137	0.000	0.075	0.000	0.103	0.000
3	0.137	0.000	0.075	0.000	0.103	0.000
4	0.137	0.000	0.075	0.000	0.104	0.001
5	0.137	0.000	0.077	0.002	0.104	0.001
6	0.137	0.000	0.075	0.000	0.103	0.000
7	0.134	0.003	0.076	0.001	0.103	0.000
8	0.138	0.001	0.071	0.004	0.103	0.000
9	0.137	0.000	0.071	0.004	0.103	0.000
10	0.137	0.000	0.076	0.001	0.103	0.000
Mean . . .	0.137 gm.	0.0005 gm.	0.075 gm.	0.0013 gm.	0.103 gm.	0.0002 gm.
$\sigma$ . . . . .	0.0010		0.00198		0.00045	
C.V. . . . .	0.72%		2.64%		0.43%	
Em. . . . .	0.00021 gm.		0.0010 gm.		0.0001 gm.	
	or		or		or	
	0.15%		1.0%		0.09%	

TABLE 7  
Mean for nine uniformly distributed samples compared with twenty-five samples

	NITRATE NITROGEN		
	Plot 5A	Plot 7A	Plot 9A
	mgm.	mgm.	mgm.
Mean of 25 samples . . . . .	0.49	0.17	0.32
Mean of 9 samples . . . . .	0.39	0.17	0.29
<i>Incubated with <math>(\text{NH}_4)_2\text{SO}_4</math> and <math>\text{CaCO}_3</math>:</i>			
Mean of 25 samples . . . . .	23.8	4.2	17.4
Mean of 9 samples . . . . .	23.4	4.14	17.0
	TOTAL NITROGEN		
	Plot 5A	Plot 7A	Plot 9A
	per cent	per cent	per cent
Mean of 25 samples . . . . .	0.138	0.076	0.104
Mean of 9 samples . . . . .	0.139	0.077	0.105

out the expected differences between the relative productivity of the three plots just as well as the mean of the twenty-five samples. In the case of the total nitrogen determinations, the mean of the nine samples varies from the mean of the twenty-five samples by only 0.001 per cent for all three plots.

The three plots under consideration in this paper also showed a correlation between their relative fertility and the amount of nitrates and total nitrogen present. While the amount of nitrates gained or lost to a soil as determined by analytical methods might appear to be minute, such amounts have a tremendous effect upon the growth of the crop. The fact that plants use nitrates directly and draw heavily upon them at certain periods of the season, makes the determination an important one. In table 8 a comparison is made between crops yields and chemical and microbiological properties of the three plots

TABLE 8  
*Comparison of crop yields as influenced by the chemical and microbiological properties of the soil*

Plot Number	TOTAL DRY MATTER PER ACRE IN CROPS FOR 15 YEARS	NITRATE NITROGEN IN UNTREATED SOIL	NITRATE NITROGEN IN $(\text{NH}_4)_2\text{SO}_4$ -TREATED SOIL	TOTAL NITROGEN CONTENT	TOTAL CARBON CONTENT	MICROORGANISMS PER G.M. OF SOIL
	lbs.	mgm.	mgm.	per cent	per cent	number
5A	70,191	0.49	23.8	0.138	1.44	11,720,000
7A	15,774	0.17	4.2	0.076	0.93	4,150,000
9A	57,848	0.32	17.4	0.104	1.13	10,130,000

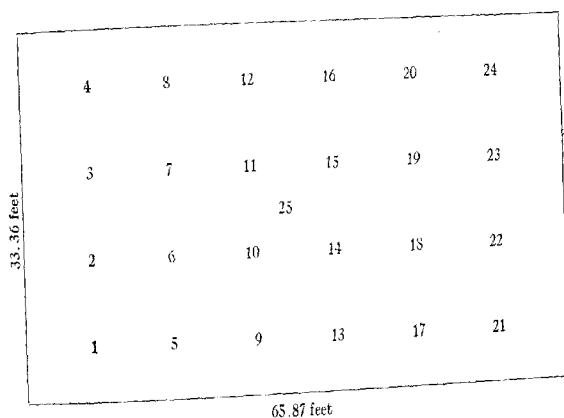


FIG. 1. DIAGRAM SHOWING LOCATION OF SAMPLING POINTS

under consideration. The numbers of microorganisms for these three plots have been recently determined by Waksman (7) from whose work the counts in table 8 have been taken. The relative fertility of these plots as measured by crop yields appears in these instances to be directly proportional to the nitrates, nitrifying power, total nitrogen and carbon content, and the number of microorganisms present. Although the nitrate content varies considerably throughout the season (2), the variations between the plots are proportionate.

## CONCLUSIONS

From this brief work, and the work of other investigators, it appears to the writer that for chemical determinations such as total nitrogen, phosphorus and potash on soil composites, where the accuracy of the analytical method is not extremely delicate and the variability of the soil is not pronounced, that nine or ten samples covering an area of one-twentieth of an acre and made into a composite would be sufficient. Duplicate or triplicate determinations on such a composite should yield results representative of the area, within the limits of the accuracy of the methods. However, when the soil constituents to be determined are changing rapidly under field conditions due to microbiological processes or to activities of plant growth, and when the method of analysis is sensitive as in the case of nitrates, a much larger number of samples must be employed to secure results that will fairly represent the area. To minimize the amount of work involved in such a process, the individual samples should be composited into several groups and these analyzed separately. The mean of such determinations would be sufficiently accurate and reliable for most practical purposes. Of course, if the coefficient of variability for a particular area has already been determined, a much smaller number of samples may be used, since the limits of variation for the area are known.

Whether a very large or relatively small number of borings should be taken in the sampling of a particular area, depends in the first place upon what the experimental data is intended to show. If comparisons are to be made between systems that have wide differences, and it is the purpose to show such differences in an approximate manner, then the number of samples need not be so great. Thus, for instance, soils from plots under different nitrogenous fertilizer treatments, would vary widely in their nitrate content and to some extent in total nitrogen. This has been shown in the work of Blair and Prince (2) on the plots at the New Jersey Experiment Station. However, where the differences between the systems under comparison are small the chance of variation between the samples within one system will be as great as the variability of the systems compared, unless the analytical methods are very delicate.

## SUMMARY

1. Variability in soils due to method of sampling was studied using nitrate and total nitrogen determinations as criteria.
2. Soil from three plots that receive different fertilizer treatment was used and twenty-five samples from each plot were taken covering an area one-twentieth of an acre in size.
3. The determinations made were nitrates on the original soil, nitrates on samples incubated with ammonium sulfate, and total nitrogen.
4. In the interpretation of the results, statistical methods were applied.
5. The crop yields from the three plots were compared and correlated with the amount of nitrate, total nitrogen content, number of microorganisms, and

6. The coefficient of variability on all three plots was very great as regards nitrates and nitrification studies, ranging between 13 and 42 per cent. The probable errors of the mean in these cases were also high, ranging from 2 to 5 per cent.

7. There was a tendency for samples of soil low in nitrate also to be low in nitrifying power. There was a similar correlation in samples high in nitrate.

8. The mean of nine composite samples covering one-twentieth of an acre was in quite close agreement with the mean of twenty-five individual samples covering the same area.

9. In the case of the total nitrogen determinations, the coefficient of variability was relatively low, being about 5.5 per cent for each plot. The probable error of the mean in each case was about 0.7 per cent. Composite samples showed no variation from the mean of a large number of individual samples. Evidently, the method for determining total nitrogen is not sensitive enough to require more than one well composited sample of soil for an area one-twentieth of an acre.

10. In order to determine how many samples of soil should be taken from a given area, three questions must first be considered: (a) Is the experimental data to show large, or small differences between the systems involved? (b) How accurate are the analytical methods? (c) Are the particular substances to be measured influenced appreciably by microbiological activities and by assimilative processes of plants?

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# COMPARISON OF "ACTIVE" ALUMINUM AND HYDROGEN-ION CONCENTRATIONS OF WIDELY SEPARATED ACID SOILS<sup>1</sup>

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In a previous article (1), a method for the determination of "active" aluminum in soils was proposed, together with reasons for its adoption. The fact that soluble aluminum salts in acid soils are often largely responsible for decreased crop yields has been shown recently by several investigators<sup>2</sup> and the desirability of ascertaining roughly the extent of geographical distribution of such active soil aluminum, as well as whether or not a correlation exists between it and soil reaction, irrespective of locality, is apparent.

A large number of active aluminum and hydrogen-ion determinations have been made on the differently treated plot soils of this station, and in the paper above cited, a fairly close correlation was shown to exist between the two; i.e., the more acid soils carried the larger amounts of soluble aluminum. Some time ago, in an endeavor to extend our knowledge along this line, the writer secured through the courtesy of a number of experiment station workers, about twenty-five samples of representative acid, mineral soils from different sections of the United States and Hawaii. Active aluminum (soluble in 0.5N acetic acid), hydrogen-ion concentration (electrometric method), and certain other characters were determined. The laboratory numbers, the localities from whence the samples were taken and brief descriptions of each follow:

1. Rhode Island. Miami silt loam.<sup>3</sup> This soil came from plot 84 of the permanent field experiments of the Rhode Island Agricultural Experiment Station, is of old glacial origin, and is typical of the acid, granitic soils of the state. So far as is known, it has never received either fertilizer or lime.

2. Maine. This soil has not been mapped by the Bureau of Soils. It was taken from the Highmoor Farm of the Maine Agricultural Experiment Station, located at Monmouth

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<sup>1</sup> Contribution 299 from the Agricultural Experiment Station of the Rhode Island State College, at Kingston.

<sup>2</sup> See bibliographies appended to the papers referred to in (1).

<sup>3</sup> Most of the soils used have been mapped, mechanically analyzed and named by the Federal Bureau of Soils. Descriptions may be found in their "Field Operations," 1899 to date.

(Kennebec County). It is a reddish-yellow silt loam, carrying considerable fine sand, and is underlaid by a compact yellow subsoil. Tile drainage is found to be decidedly beneficial in certain localities, especially for orchard work. It is of early glacial origin.

3. Pennsylvania. Volusa silt loam. This sample came from the Bradford County Experimental Plots of the Pennsylvania State Agricultural Experiment Station. The Volusa soils are found in the northern part of Pennsylvania and in New York State. They are normally very acid and poorly drained and represent the only glaciated soils (later Wisconsin drift) to be found in Pennsylvania. They owe their origin to the glaciation of the underlying country rock, with the addition of some foreign material. No lime had ever been applied to the area from which this sample was drawn.

4. Pennsylvania. DeKalb silt loam. This soil came from a check plot of the Snow Shoe Experimental Fields of the Pennsylvania State Agricultural Experiment Station, located in

TABLE 1  
"Active" aluminum, hydrogen-ion concentration and other data on soils from different parts of the United States

SOIL NUMBER	LOCATION	SOIL TYPE	REACTION	ACTIVE $\text{Al}_2\text{O}_3$ IN DRY SOIL	LOSE ON IGNITION	NITROGEN	SILT*	CLAY*
			pH	p.p.m.	per cent	per cent	per cent	per cent
1	R. I.	Miami silt loam	4.47	819	5.7	0.137	65	15
2	Me.	Reddish-yellow silt loam	4.90	662	16.6	0.208	50	18
3	Pa.	Volusa silt loam	4.45	270	11.4	0.264	61	23
4	Pa.	DeKalb silt loam	4.17	300	9.2	0.132	56	19
5	Pa.	Hagerstown clay loam	4.17	294	7.4	0.155	59	30
6	S. Car.	Portsmouth sandy loam	5.77	42	10.6	0.201	8	5
7	S. Car.	Greenville clay loam	5.70	72	4.2	0.041	14	33
8	Ky.	Decatur silt loam	4.50	128	6.3	0.126	63	20
9	Ky.	Memphis silt loam	4.72	44	4.9	0.087	83	9
10	Ind.	Wakasha silt loam	4.90	63	9.7	0.236	52	14
11	Ind.	Clermont silt loam	5.15	48	4.3	0.080	53	27
12	Ind.	Scottsburg silt loam	5.22	50	4.4	0.093	62	10
13	Ill.	Upland prairie brown silt loam	5.58	19	11.1	0.268	70	18
14	Iowa	Carrington loam	5.20	Trace	11.4	0.252	43	22
15	N. D.	Fargo clay	5.66	None	24.3	0.425	38	46
16	Tex.	Lufkin fine sandy loam	5.31	None	8.8	0.118	16	7
17	Tex.	Subsoil of No. 16	5.30	Trace	4.2	0.077	15	41
18	Ore.	Melbourne silty clay loam	4.50	890	16.6	0.285	42	43
19	Ore.	Medium sandy loam	4.63	657	26.6	0.397		
20	Cal.	"No. 13" fine sandy loam	4.00	53	3.3	0.062		
21	Cal.	"No. 23" fine sandy loam	4.63	60	3.5	0.072		
22†	Hawaii	Hawaii brown clay loam. †	5.31	1820	45.4	0.699		
23	Hawaii	Oahu red clay loam	4.65	537	25.7	0.349		
24	Hawaii	Kauai black clay loam	4.60	660	25.6	0.395		

\* U. S. Bureau of Soils Reports. Percentages given are taken from analyses of soils from the state and usually from the county from which the above samples were drawn.

† This soil has been omitted from all averages, due to its extremely high content of soluble aluminum, organic matter and nitrogen.

Centre County, and represents forty-three per cent of the soil area of the state. It is a residual soil, occurring only in non-glaciated sections. While often acid and in a depleted condition, it responds well to fertilizers and lime and may often be profitably reclaimed.

5. Pennsylvania. Hagerstown clay loam. This sample was taken from plot 32 of the old fertilizer experiments at the State College (Centre County) Pennsylvania, and has been treated for forty years with ammonium sulfate, acid phosphate and muriate of potash. It occurs in a non-glacial area and was originally derived from limestone.

6. South Carolina. Portsmouth sandy loam. This sample was drawn near Georgetown (Georgetown County) and represents a coastal plain type of very poor, acid soil. It is low-lying, flat land of little agricultural value except for pasturage.

7. South Carolina. Greenville clay loam. This soil is also a coastal plain type coming from Trenton (Edgefield County).

8. Kentucky. Decatur silt loam. This sample came from the Russellville Experiment Field, Logan County, and is typical of considerable areas in central Kentucky. It was drawn from a non-glaciated section and is of limestone origin.

9. Kentucky. Memphis silt loam. This soil is from the Mayfield Experiment Field, located in Graves County, and is of loessial origin. It is fairly representative of western Kentucky and areas along the Mississippi River.

10. Indiana. Wakasha silt loam. This sample of soil was drawn from the Indiana Agricultural Experiment Station farm at West Lafayette (Tippecanoe County) Indiana. This type rests upon the late Wisconsin drift, and consists of glacial material, reworked and laid down by water. It does not respond to lime treatments.

11. Indiana. Clermont silt loam. This sample was taken from an untreated plot of the Jennings County Experiment Field of the Indiana Agricultural Experiment Station, located near Vernon. This is a very light colored soil (nearly white) and is of glacial origin.

12. Indiana. Scottsburg silt loam. This soil came from the check plot of the Scottsburg Experiment Field (Scott County) of the Indiana Agricultural Experiment Station. As it occurs within the Illinoian drift area, a considerable part of the material from which this soil has been formed was deposited through glacial action. This soil responds well to lime and soluble phosphates.

13. Illinois. Upland prairie brown silt loam (2). This sample was secured from the farm of D. Smith, about three miles east of Urbana (Champaign County), Illinois, and is a typical, rich corn-belt soil. So far as is known, it has not received lime or commercial fertilizers of any kind. It is composed of fine material of loessial character from the Champaign till sheet of the early Wisconsin glaciation.

14. Iowa. Carrington loam. This soil was drawn from the experiment station farm of the Iowa Agricultural Experiment Station at Ames (Story County). It is a mellow, black soil, usually of high productivity. The material was originally formed by glacial action (Kansan drift).

15. North Dakota. Fargo clay. This sample was drawn from the old wheat plot (plot 2 in rotation series 1) of the North Dakota Agricultural Experiment Station, Agricultural College (Cass County), North Dakota. This soil has now grown forty consecutive crops of wheat.

16. Texas. Lufkin fine sandy loam. This soil, together with no. 17, which is the subsoil, was taken from the farm of the Texas Agricultural Experiment Station, College Station (Brazos County), and is the most widely distributed type of this series, occupying large areas in this section of Texas. It is flat or slightly rolling and is derived largely from grayish or drab clays and grayish, argillaceous sands. Unaltered clays and sands usually appear at about 5 feet.

17. Texas. Subsoil of no. 16.

18. Oregon. Melbourne silty clay loam. This sample was taken two miles west of Alpine in Benton County. It represents a reddish soil which prevails in comparatively large areas. It responds to lime applications.



19. Oregon. This soil has not been surveyed by the Federal Bureau of Soils but is classified by the Agronomy Department of the Oregon Agricultural College as a medium sandy loam. It was obtained a few miles north of Nahalem in Clatsop County.

20. California. "Soil no. 13." This is a fine sandy loam from the coast of Mendocino County, and is very light gray in color. It is not yet mapped by the Federal Bureau of Soils. It is probably of volcanic origin.

21. California. "Soil no. 23." This sample came from near Santa Rosa, Sonoma County, and is much improved by lime. It has not been mapped.

22. Hawaiian Islands, Hawaii. This soil came from one of the upper fields of the Honokaa Sugar Company (section 37A field 5) which is located on the windward side of the Island of Hawaii in the very heavy rainfall belt. This soil is subjected to excessive leaching. It is a dark brown clay loam. All of the Hawaiian soils here discussed are derived entirely from volcanic rock of fairly recent geologic date, and would be classed as laterites.

23. Hawaiian Islands. Oahu. This sample was drawn in the Kailua District on the Island of Oahu, from the pineapple fields of Libby, McNeil & Libby (Field no. 18). It comes from an elevation of approximately 400 feet and is in a section of fairly heavy rainfall. The soil is a brick-red clay loam, and carries large quantities of the oxides of iron, aluminum and some manganese.

24. Hawaiian Islands. Kauai. This soil came from one of the upper fields of the Koloa Sugar Company (section 6, field 54) on the Island of Kauai. The rainfall is fairly heavy. It is a black clay loam soil well supplied with organic matter.

A perusal of table 1 brings out several interesting points. At first it appears that no relationship whatever exists between the hydrogen-ion concentrations of soils of different types and the amounts of active aluminum contained therein. For instance, the most acid soil reported, no. 20 from California, with a reaction of pH 4.00, yields but 53 p.p.m. of alumina soluble in 0.5 *N* acetic acid, while the least acid one, no. 6 from South Carolina, with a reaction of pH 5.77, gives an amount of similar magnitude. As a rule, however, the soils whose pH values are less than 5; (i.e., the more acid soils,) carry much larger amounts of active aluminum than do those whose pH values are greater than pH 5. The average for the former group is 388 p.p.m. of alumina, while for the latter it is but 26 p.p.m. Thus while the acidities of the different soils may not always be directly correlated with the amounts of active aluminum found, the group averages are so correlated, as are the individual soils of varying reaction *within the same soil type*. Here a fairly close relationship between the two exists unless unusually large applications of acid phosphate or lime have been recently made. This fact has been indicated in my first paper (1) and is still more definitely shown by unpublished data soon to appear.

Another factor which tends to interfere with such a correlation between soils of different types as well as between those of the same type is organic matter. In an endeavor to shed some light on the effect of organic matter on aluminum solubility in acid soils, "loss on ignition" determinations were made. It is well understood, of course, that this loss in weight would include water of hydration or combination and possibly ammonium salts (no carbonates could exist in these acid soils) besides organic matter, but the latter would most certainly account for by far the larger portion of the losses re-

corded. There appears to be no definite correlation between the percentages of organic matter in the soils and the amounts of active aluminum, although they often vary in the same direction; i.e., the larger the loss on ignition, the greater the amounts of soluble aluminum. Just the reverse of the above finding has been shown to obtain in soils of the *same type* (1). Here large applications of organic matter appeared to decrease the amounts of readily soluble aluminum, although it should be borne in mind that the latter materials were barnyard or green manures of a readily decomposable nature, whereas in the soils reported in table 1, much of the organic matter was undoubtedly old, difficultly decomposable and but slightly soluble. As would be anticipated, the percentages of nitrogen in these soils vary with the organic matter present.

It has long been recognized that the soil materials dissolved in the soil solution, or readily soluble in an excess of any solvent, are derived almost exclusively from the finer soil separates; i.e., the silt and the clay. As clay is largely made up of hydrated aluminum silicates, it seemed of importance to ascertain whether any correlation existed between the readily soluble aluminum of acid soils and the percentages of their finer mechanical fractions. The last two columns of table 1 show the approximate percentages of silt and clay in a majority of the soils under discussion. No relationship is evident between either the hydrogen-ion concentrations or the "active" aluminum contents and the percentages of these mechanical fractions.

A careful perusal of the rainfall data for the sections from which these samples came was made by the writer where possible and it appears that in a great majority of cases, the heavier the rainfall, the larger were the amounts of active aluminum found.

The factor chiefly responsible for the presence of readily soluble aluminum in acid soils is doubtless to be found in soil genesis. Unfortunately, quantitative mineralogical analyses have not been made on the soil samples under discussion. With the exception of the laterites from the Hawaiian Islands, the glacial soils carry much larger amounts of soluble alumina than do those from the unglaciated regions, the older glaciations exceeding the more recent in this respect. This latter fact is in agreement with Slipher (3) who states, "The older the glacial age of soils, the higher the response to lime treatments. . . . The oldest glaciation has furnished the largest crop response. With the exception of the Iowan, the apparent need for lime becomes progressively less with youth."

An important fact indicated by the data here presented is that the degree or amount of soil acidity (either hydrogen-ion concentration or "lime requirement") does not necessarily indicate the amounts of active aluminum which may be present, which may explain why certain acid soils respond but feebly to lime applications. Data to be published elsewhere will show that lime unaccompanied by soluble phosphate is occasionally required in large quantities to depress adequately aluminum solubility and hence toxicity, in these cases. The idea must not be gained that ordinary lime applications are gener-

ally futile in rendering active aluminum innocuous where the latter is present in limited amounts and where the physical condition of the soil is good, but even here soluble phosphates as well as lime are advocated for quick action.

#### SUMMARY

About twenty-five samples of representative acid, mineral soils were secured from as many widely separated sections of the United States, including the Territory of Hawaii. "Active" aluminum determinations by a method recently proposed by the writer (1), and hydrogen-ion determinations by the electrometric method were made, as were also "loss on ignition" and total nitrogen determinations. From these data the following conclusions seem warranted.

Considered from the point of view of a mass average, a direct correlation exists between hydrogen-ion concentration and active aluminum in acid soils, although there are several individual exceptions. The more acid soils (pH 4 to 5) averaged 388 p.p.m. of active alumina while the less acid group (pH 5 to 5.8) averaged but 26 p.p.m. (dry soil basis). The exceptions are of interest in that high active aluminum and low acidity may at times explain a lack of response to liming, for we have data which show that both lime and soluble phosphate applications are needed to depress greatly the activity of aluminum in certain acid soils.

No definite correlation was noted between "active" aluminum and the percentages of either organic matter, nitrogen, clay or silt, although often the soils rich in organic materials (and also in nitrogen) yielded the larger amounts of soluble aluminum.

A casual relationship appeared to exist between rainfall (and hence leaching) and "active" aluminum.

Soil genesis is probably largely responsible for the differences recorded in the data here presented. With the exception of the Hawaiian laterites, the glaciated soils carried much larger amounts of active aluminum than do the others, the older exceeding the more recent in this respect.

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# THE INFLUENCE OF SOLUTION VOLUME UPON PLANT GROWTH IN RELATION TO REACTION CHANGE AND IRON AVAIL- ABILITY IN CULTURE SOLUTIONS<sup>1</sup>

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## INTRODUCTION

The nature and the rates of plant growth in culture solutions are determined by many factors. The relation of some of these factors to plant development is quite intricate and not at all well understood while that of others appears at first so simple as not to require much consideration. One of these apparently simple factors involved in all solution culture studies, that of the relation of solution volume or size of the culture vessel to the various types of plant activity, has, perhaps, not received the attention which its importance merits.

In some preliminary experiments dealing with the rate of change of hydrogen-ion concentration brought about by the action of growing wheat plants in two different types of culture solutions and the effect of this change upon iron availability (1) it was found that the solution volume had a marked influence not only upon the character and rates of growth directly but also upon the reaction change produced in the culture solutions and upon the manner in which the plants responded towards different compounds used as sources of iron for the plants. The phenomena observed in connection with this preliminary study appeared to be of sufficient importance to warrant further investigation.

The experiments reported in this paper were undertaken primarily for the purpose of investigating the effect of different solution volumes upon the growth rates and development of young wheat plants in two types of culture solutions. A study was also made of the hydrogen-ion concentration changes brought about by the action of the plants in the different solution volumes and of the influence of these changes upon the availability of iron added to the solutions in a soluble and an insoluble form.

## METHODS OF PROCEDURE

The culture solutions used in these experiments were Tottingham's (8) four-salt solution  $T_1R_1C_3$  and a modification of this solution used by Jones

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and Shive (5). This modification consisted in substituting ammonium sulfate for the potassium nitrate of the Tottingham solution in equivalent osmotic concentrations. Baker's analyzed salts were used in the preparation of half molecular stock solutions from which the culture solutions were prepared. These were made up to have a calculated total osmotic concentration value of one atmosphere. The proportions of the salts in volume-molecular partial concentrations are given in table 1.

Iron was added to the culture solutions in the forms of ferric phosphate and ferrous sulfate. The ferric phosphate was prepared as described by Jones and Shive (4) while the ferrous sulfate was used in aqueous solution always freshly prepared just before being added to the culture solutions.

The culture vessels consisted of wide-mouth bottles having capacities of 250, 500, 1000, and 2000 cc. Wheat of the "Marquis" variety was germinated on a germinating net (7) and uniform seedlings were carefully selected, mounted

TABLE 1  
*Volume-molecular partial concentrations of the salts in the two types of culture solutions used*

TYPE OF SOLUTION	VOLUME-MOLECULAR PARTIAL CONCENTRATIONS				
	KNO <sub>3</sub>	Ca(NO <sub>3</sub> ) <sub>2</sub>	MgSO <sub>4</sub>	KH <sub>2</sub> PO <sub>4</sub>	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>
Tottingham T <sub>1</sub> R <sub>1</sub> C <sub>3</sub> .....	0.0020	0.00438	0.01185	0.00211	
Modified Tottingham T <sub>1</sub> R <sub>1</sub> C <sub>3</sub> .....		0.00438	0.01185	0.00211	0.0014

in the double-piece paraffined cork stopper devised by Tottingham (8) and were then transferred to the culture solutions.

The culture solutions were renewed every three and one-half to four days. Each period between two successive solution changes will be designated as a growth interval. After each solution change, the old solutions were made up to their original volumes with distilled water and samples removed for the determination of hydrogen-ion concentrations which were recorded in terms of pH values. These determinations were made colorimetrically by the use of the double-tube standards of Gillespie (3), the indicators recommended by Clark (2), and with the apparatus devised by Van Alstine (9).

Three series of cultures were conducted in each of which wheat plants were grown in both the Tottingham solution T<sub>1</sub>R<sub>1</sub>C<sub>3</sub> and in the ammonium sulfate modification of this solution in the four different solution volumes (250, 500, 1000, and 2000 cc.) and the cultures were duplicated. The respective series are further described in connection with the presentation of the experimental data.

## EXPERIMENTAL RESULTS

### *Series 1*

In the cultures comprised in this series two wheat plants per culture were grown in the different volumes of the two types of culture solutions described.

The cultures were begun on March 4 and grown for a period of five weeks. Iron for the plants of this series was supplied in the form of ferrous sulfate in quantities of 1 mgm. of iron per liter in the Tottingham solution and 0.05 mgm. in the ammonium sulfate modification of this solution. These amounts were found by Jones and Shive (5) to give satisfactory results in the respective solutions during the early stages of the growth of wheat.

At the end of the growth period of five weeks the plants were harvested and the dry weights of tops and roots determined separately. The average dry weights of tops and of roots per plant and the average total dry weights per plant together with the hydrogen-ion concentrations of the solutions determined at the end of each growth interval are given in table 2.

TABLE 2  
*Dry weights of plants and pH values of solutions at the end of each growth interval*

SOLUTION VOLUME	AVERAGE DRY WEIGHT PER PLANT			REACTIONS OF SOLUTIONS AT END OF INTERVALS*									
	Tops	Roots	Total	1	2	3	4	5	6	7	8	9	10
	gm.	gm.	gm.	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH
<i>Tottingham's solution <math>TiR_2C_4</math>, 1.0 mgm. of iron (<math>FeSO_4</math>) per liter*</i>													
2000	1.5998	0.3027	1.9025	4.9	4.9	5.3	5.3	5.9	6.0	6.2	6.4	6.5	6.2
1000	1.7757	0.3197	2.0954	4.9	5.3	5.7	5.7	6.3	6.2	6.6	6.7	6.9	7.1
500	1.7435	0.3489	2.0927	5.5	5.5	5.8	6.2	6.7	6.7	7.0	7.3	7.5	7.3
250	1.1422	0.2835	1.4257	5.6	5.8	6.0	6.4	7.0	7.0	7.2	7.2	7.2	7.8
<i>Modified Tottingham's solution <math>TiR_2C_5</math>, 0.05 mgm. of iron (<math>FeSO_4</math>) per liter*</i>													
2000	2.5904	0.5014	3.0918	4.5	4.7	4.7	4.7	4.6	4.5	4.6	4.6	4.5	6.5
1000	2.3314	0.3832	2.7146	4.5	4.6	4.4	4.6	4.5	4.4	4.5	4.5	4.3	6.7
500	1.6885	0.3083	1.9968	4.5	4.4	4.3	4.4	4.4	4.1	4.5	5.7	5.0	7.2
250	1.1693	0.2684	1.4376	5.9	4.5	4.1	4.3	4.4	4.3	5.9	7.0	6.9	7.4

\* Initial pH value of both types of solution approximately 4.7.

It will be observed from the data of table 2, that the different volumes of the Tottingham solution, except the smallest, (250 cc.), produced yields which are nearly equal in numerical value. In the 250-cc. cultures, however, growth was considerably depressed. The average dry weights of tops and of roots produced by the different volumes of the modified solution containing ammonium sulfate, on the other hand, are highest for the 2000-cc. cultures and are progressively lower for the cultures with smaller solution volumes, the lowest yields being obtained from the 250-cc. cultures. It is interesting to note also that the solution containing ammonium sulfate proved to be a better medium than the unmodified Tottingham solution for the growth of these plants in the larger volumes while in the smaller volumes (250 and 500 cc.) the two types of solutions produced practically the same yields of both tops and roots.

The pH values of these solutions determined at the end of the growth intervals are presented graphically in figure 1. The upper set of four graphs in this figure represents the pH values for the four different volumes of the Tottingham solution after contact with the roots of the growing plants, while the lower set represents in a like manner the pH values of the four different volumes of the modified Tottingham solution containing ammonium sulfate.

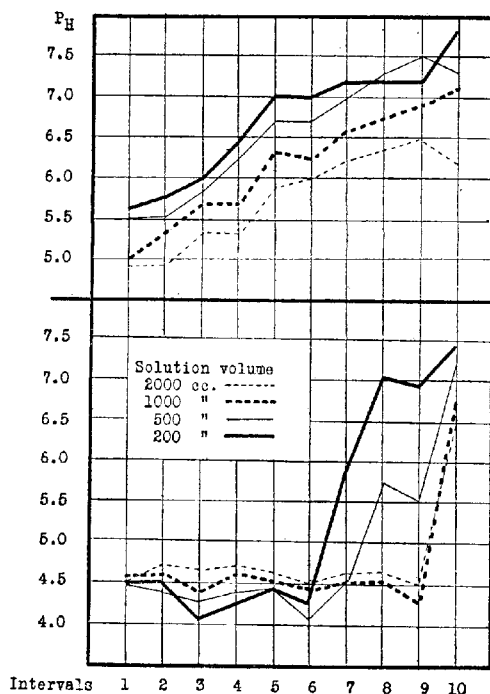


FIG. 1. GRAPHS OF pH VALUES OF CULTURE SOLUTIONS AFTER CONTACT WITH PLANT ROOTS DURING THE VARIOUS GROWTH INTERVALS

Upper graphs, Tottingham's solution  $T_1R_1C_3$ ; lower graphs, modified solution containing ammonium sulfate.

The graphs of pH values of the Tottingham solution in this series of cultures show a gradual decrease in hydrogen-ion concentrations at the end of the growth intervals, as the plants become older, in each of the four different volumes of solution. However, at the end of any interval the greatest reaction change is always shown for the smallest volume and the least reaction change for the greatest volume. This indicates that while the rates of reac-

tion change increase with the age of the plants in each volume of solution the pH values at any time during the growth period are by no means independent of the volume of the solution in which the plants are growing.

With respect to change of reaction, results quite different from those shown for the Tottingham solution were obtained with the modified Tottingham solution containing ammonium sulfate as a comparison of the upper with the lower set of graphs in figure 1 clearly demonstrates. A slight increase in hydrogen-ion concentration over the initial concentration is observed during the first six growth intervals in each of the four different volumes of solution, the magnitude of reaction change nearly always being greatest in the smallest volume and least in the largest volume of solution. During the seventh interval, however, there was a marked decrease in the hydrogen-ion concentrations of the solutions of the 250-cc. and the 500-cc. cultures. This reversal in the direction of reaction change is shown in the abrupt rise in the graphs representing the pH values of the solutions of these two cultures in the seventh interval. At the end of the ninth interval a similar break is observed in the two graphs representing the pH values of the solutions of the 1000-cc. and the 2000-cc. cultures.

This reversal in the direction of reaction change shown by the graphs may indicate a very important stage in the development of the plants with respect to the requirements by the plants for certain nutritive ions the selective absorption of which has a pronounced influence upon the reaction of the nutrient solutions. Prince, Jones, and Shive (6) have suggested that in culture solutions containing ammonium sulfate and calcium nitrate such as were here used, the direction of reaction change is determined, in a large measure, by the relative rates of absorption by the plants of the  $\text{NH}_4$ -ions and the  $\text{NO}_3$ -ions (these always being absorbed at a relatively much higher rate than are the corresponding equivalent, oppositely charged  $\text{SO}_4$ -ions and Ca-ions, respectively) the selective absorption of the former tending to increase, and that of the latter to decrease the hydrogen-ion concentrations of the culture solutions. They state that during the early stages of growth the soybean plants used in their experiments drew heavily upon the  $\text{NH}_4$ -ions and less heavily upon the  $\text{NO}_3$ -ions but that this condition was reversed at a later stage of development and was accompanied by a reversal in the direction of reaction change. These suggestions are based on careful quantitative chemical analyses of the culture solutions after being in contact with the roots of the actively growing plants for definite time intervals. Assuming that these suggestions define in a general way the conditions which actually exist in the solutions here used with respect to the absorption by the wheat plants of the ions in question, it is at once clear from the graphs of figure 1 that the decrease in the hydrogen-ion concentration of the cultures solutions as indicated by the abrupt rise in the graphs representing the pH values may be the result of a pronounced physiological change in the plants with respect to the ammonium and nitrate requirement involving a change in the absorption rates of these ions which completely reverses the direction of reaction change of the culture solutions.



The influence of volume upon the reaction change of the culture solutions is clearly shown by the graphs. As previously indicated, the magnitude of reaction change always varies in the inverse order of the volumes of the culture solutions. Since any alteration in the proportions of the ions in the culture solutions brought about by selective absorption, must take place much more slowly in the larger volumes than in the smaller ones, any pronounced reaction change resulting from such absorption must, of course, be correspondingly delayed in the larger solution-volumes. It is thus observed that the marked reaction change of the solutions of the 250-cc. and the 500-cc. cultures comes at the end of the sixth interval and that of the solutions of the 1000-cc. and the 2000-cc. cultures at the end of the ninth interval as is indicated by the graphs of pH values.

### Series 2

As previously stated, series 2 was like series 1 except that iron in the form of ferric phosphate was added to the solutions in quantities of 5.0 mgm. per

TABLE 3  
*Dry weights of plants and pH values of solutions at the end of each growth interval*

SOLUTION VOLUME	AVERAGE DRY WEIGHT PER PLANT			REACTION OF SOLUTIONS AT END OF INTERVALS*									
	Tops	Roots	Total	1	2	3	4	5	6	7	8	9	10
				pH	pH	pH	pH	pH	pH	pH	pH	pH	pH
cc.	gm.	gm.	gm.										
<i>Tottingham's solution <math>T_1R_2C_3</math>, 5.0 mgm. of iron (<math>FePO_4</math>) per liter*</i>													
2000	2.9540	0.5818	3.5358	4.7	4.7	5.0	5.2	5.5	5.7	6.1	6.2	6.5	6.4
1000	2.2626	0.4182	2.6808	4.8	5.0	5.3	5.4	6.0	6.0	6.4	6.6	6.7	6.7
500	1.7371	0.3810	2.1181	5.0	5.2	5.6	5.7	6.2	6.4	6.7	7.0	7.1	7.2
250	1.2365	0.2988	1.5353	5.3	5.6	6.0	6.3	6.8	7.0	7.1	7.1	7.1	7.3
<i>Modified Tottingham's solution <math>T_1R_2C_3</math>, 5.0 mgm. of iron (<math>FePO_4</math>) per liter*</i>													
2000	3.5001	0.5329	4.0330	4.5	4.6	4.6	4.6	4.7	4.5	4.5	4.5	4.5	5.0
1000	3.0749	0.4856	3.5605	4.4	4.6	4.4	4.5	4.5	4.5	4.3	4.5	5.3	6.1
500	2.6768	0.4519	3.1287	4.7	4.6	4.4	4.4	4.4	4.5	5.0	5.2	5.9	7.2
250	2.1604	0.3591	2.5196	4.9	4.6	4.1	4.3	4.3	5.0	4.8	6.5	7.2	7.1

\* Initial pH values of both types of solutions approximately 4.7.

liter in all the cultures. However, only one plant was grown in each culture in order to study the reaction change in the different volumes of solution when the rate of alteration in the proportions of the salt constituents due to absorption by the plants was reduced to a minimum under these cultural conditions. The cultures were duplicated and this series was conducted simultaneously with series 1. The numerical data are presented in table 3 and these correspond in every way with those given in table 2. The pH values as given in table 3 are shown graphically in figure 2. These graphs were prepared in the same manner as were those in figure 1 representing the pH values of the culture solutions used in series 1.

The relations with respect to reaction change produced in the culture solutions by the action of the growing plants as indicated by the graphs of figure 2, are in general quite the same as are those shown by the graphs of figure 1. This is clearly apparent from the close resemblance and the almost perfect agreement between the corresponding sets of graphs. A comparison of the graphs and of the data of pH values in tables 2 and 3 brings out the fact that the magnitude of reaction change produced by a single plant in the different volumes of solution is, with few exceptions, practically as great as that pro-

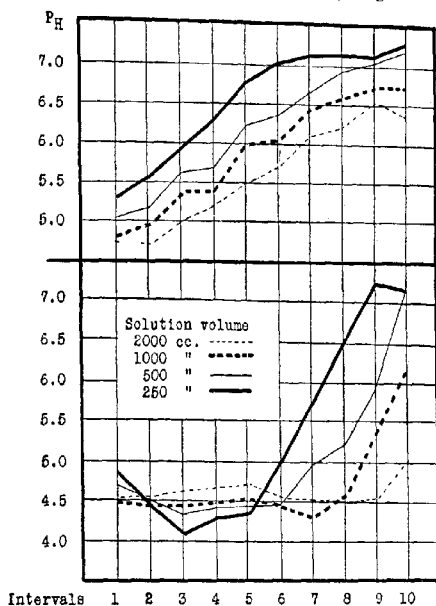


FIG. 2. GRAPHS OF pH VALUES OF CULTURE SOLUTIONS AFTER CONTACT WITH PLANT ROOTS DURING THE VARIOUS GROWTH INTERVALS

Upper graphs, Tottingham's solution  $T_1R_1C_3$ ; lower graphs, modified solution containing ammonium sulfate.

duced by the two plants in corresponding solution-volumes. This is not surprising, however, when it is observed from a comparison of the yield data of tables 2 and 3 that the average dry weight per plant from the one-plant cultures is always much greater than that from the corresponding two-plant cultures, the greater size and vigor of the plants from the former influencing the reaction change of the culture solutions correspondingly.

The pH values of the modified Tottingham solution containing ammonium sulfate are here again slightly reduced below the initial values by the action of the plants during the first five growth intervals, after which the direction

of the reaction change is reversed but not quite so abruptly as in the corresponding solutions of the two-plant cultures of series 1. As indicated by the graphs this change in the direction of reaction from lower to higher pH values occurs first in the solutions of the 250-cc. cultures and is followed in order in the larger solution volumes occurring last in the solutions of the 2000-cc. cultures, the relative magnitude of the reaction change being determined always by the volume of the culture solution.

Examination of the dry weight data of table 3 brings out the fact that even with a single plant in each culture the growth rates are greatly retarded in the smaller solution volumes of both types of culture solutions, the average dry weight per plant from the 2000-cc. cultures being nearly double that from the 250-cc. cultures. It is also observed that the solution containing ammonium sulfate produced larger yields in each of the four volumes than did the Tottingham solution in corresponding volumes. The total average dry weight per plant in the solution containing ammonium sulfate was 14.0 per cent higher for the 2000-cc. cultures and 64.0 per cent higher for the 250-cc. cultures than were those in the corresponding volumes of the Tottingham solution, the superiority in yield being progressively less in passing from the smaller to the larger volumes. The explanation of this appears in the fact that whenever by the action of the plants the hydrogen-ion concentrations of the solutions were reduced to pH values above 6.0 the plants thereafter became chlorotic and their growth rates were checked. This condition became quite pronounced in all the plants grown in the Tottingham solution in both series 1 and 2 but did not occur at all in the plants grown in the solutions containing ammonium sulfate except to a slight degree in the plants of the 250-cc. and the 500-cc. cultures at the end of the growth period.

In both types of culture solutions there is a direct correlation shown between the reaction of the medium, determined at the end of the growth intervals, and the appearance of chlorosis in the plants thus indicating that the availability to the plants of the form of iron here used is determined by the reaction of the solution.

### *Series 3*

This series was carried out for the purpose of determining whether results somewhat like those obtained from the cultures of the preceding series could be produced at a different season of the year under similar cultural conditions and also to determine the effect of iron in the form of ferric phosphate when supplied in equal amounts to the plants in the different solution volumes of both types of culture solutions here used. The cultures of this series were conducted from September 4 to October 31. In the preceding series this form of iron was supplied in quantities of 5.0 mgm. of iron per liter of solution. Thus the total quantity of iron per culture varied from 10.0 mgm. of iron in the 2000-cc. cultures to 1.25 mgm. in the 250-cc. cultures, the total iron supplied to the plants of the different cultures being directly proportional to the

volume of the culture solutions. The present series comprised two groups of cultures. In the first group the culture solutions were exact duplicates of those of the preceding series throughout and the solutions of the second group were like those of the first except that 10.0 mgm. of iron as ferric phosphate were added to each of the four different volumes of solution so that the total iron supplied to the plants of each culture was equal throughout.

For the sake of convenience the method of supplying iron to the solutions in the first group of cultures may be designated as iron added on the "liter basis" (5.0 mgm. of iron per liter of solution), in the second group of cultures as iron supplied on the "culture basis" (10.0 mgm. of iron per culture regardless of solution-volume). In both groups three plants were grown in each culture.

In table 4 are given the average dry weights per plant and the pH values of the solutions determined at the end of the growth intervals. Since the pH values of solutions of corresponding cultures in the two groups agreed very closely, these have been averaged and the table gives only these average values for the different solution-volumes. These data apply, therefore, to the cultures in which iron was supplied on the liter basis as well as to those in which iron was supplied on the culture basis. The pH values were plotted as in the previous series to form the two sets of graphs given in figure 3.

The graphs of figure 3 show that the relations between hydrogen-ion concentration, as this is influenced by plant action, and the volume of the culture solution in which the plants were grown are quite the same as are those shown for the preceding series. It is again observed that the graphs representing the pH values of the Tottingham solutions (upper set of graphs, figure 3) lie one above the other, the upper graph representing the pH values of the solutions of the 250-cc. cultures. Below this graph, following in the order of the magnitudes of solution volume, lie the other three graphs without intersecting at any point. In the Tottingham solution the action of the plants tends always to decrease the hydrogen-ion concentration, the rate of reaction change steadily increasing with the age of the plants during the early stages of growth.

The plants in the culture solution containing ammonium sulfate, on the other hand, tend by their action to increase the hydrogen-ion concentrations of the solution above the initial concentration, during the early stages of growth. This is again followed by a reversal in the direction of reaction change which occurred first in the 250-cc. cultures during the sixth interval, in the 500-cc. cultures during the seventh interval, and in the 1000-cc. and 2000-cc. cultures during the tenth interval. This is clearly shown by the rather abrupt upward pitch in the graphs (lower set, fig. 3) of pH values for the intervals indicated, occurring in somewhat the same manner as that shown by the corresponding graphs of pH values for the culture solutions of series 1 and 2, the relative magnitudes of change in reaction being determined always by the volume of the culture solution.

Comparing now the dry weight data of the two groups of cultures of series 3 as given in table 4, it is observed that in both types of solutions the 500-cc.

TABLE 4  
*Dry weights of plants and pH values of solutions at the end of each growth interval. Iron supplied in the form of ferric phosphate*

SOLUTION VOLUME	AVERAGE DRY WEIGHTS PER PLANT						AVERAGE REACTION OF SOLUTIONS AT END OF GROWTH INTERVALS														
	Group 1—Iron on liter basis (3.0 mgm. per liter).			Group 2—Iron on culture basis (10.0 mgm. per liter).			Total gm.	Roots gm.	Tops gm.	Total gm.	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH	
	Tops	Roots	Total	Tops	Roots	Total															
	gm.	gm.	gm.	gm.	gm.	gm.															
cc.																					
Tottingham solution T <sub>1</sub> R <sub>1</sub> C <sub>3</sub>																					
2000	3.4625	0.3758	3.8383	3.5295	0.3672	3.7005	5.05	5.35	5.05	5.25	5.85	6.10	5.90	6.35	6.30	6.45	6.25	6.30	6.35	6.75	6.75
1000	3.4600	0.4487	3.9087	3.4275	0.3915	3.8190	5.05	5.55	5.15	5.70	6.00	6.35	6.10	6.60	6.50	6.65	6.50	6.55	6.70	6.85	7.05
500	2.6152	0.3332	2.9483	3.0923	0.4467	3.5357	5.15	5.63	5.95	5.90	6.20	6.55	6.30	6.90	6.85	6.90	6.85	6.75	6.90	7.05	7.60
250	1.7852	0.3190	2.1042	2.5100	0.3225	2.8325	5.25	5.85	6.30	6.20	6.50	6.70	6.75	6.95	6.90	7.00	6.90	6.90	7.00		
Modified Tottingham solution T <sub>1</sub> R <sub>1</sub> C <sub>3</sub>																					
2000	5.2400	0.4800	5.7200	4.8782	0.4882	5.3630	4.83	5.13	4.86	4.80	4.70	4.86	4.83	5.00	4.90	5.76	5.30	5.00	5.70	5.25	5.95
1000	3.4908	0.3433	3.8341	3.0990	0.2997	3.3987	4.93	5.10	4.70	4.60	4.70	4.66	4.96	5.06	5.63	6.10	6.43	6.47	6.70	6.63	6.76
500	2.8088	0.2952	3.1040	2.8680	0.3483	3.2113	4.83	4.53	4.47	4.53	4.63	4.93	5.00	4.60	4.60	6.03	6.73	6.70	6.83	6.76	6.90
250	2.0212	0.3060	2.3272	2.2840	0.3112	2.5952	4.53	4.30	4.23	4.30	4.40	5.50	5.50	5.95	6.46	6.86	6.80	6.83	6.90	6.90	6.90

cultures and the 250-cc. cultures in which iron was supplied on the culture basis (10.0 mgm. of iron per culture) produced yields which were considerably higher than those from the corresponding cultures in which iron was supplied on the liter basis (5.0 mgm. of iron per liter), the ratios of the relative amounts of iron supplied by the former method to those supplied by the latter being as 4:1 and 8:1, respectively. Thus in the smaller solution volumes the application of these larger amounts of iron resulted in somewhat higher yields owing

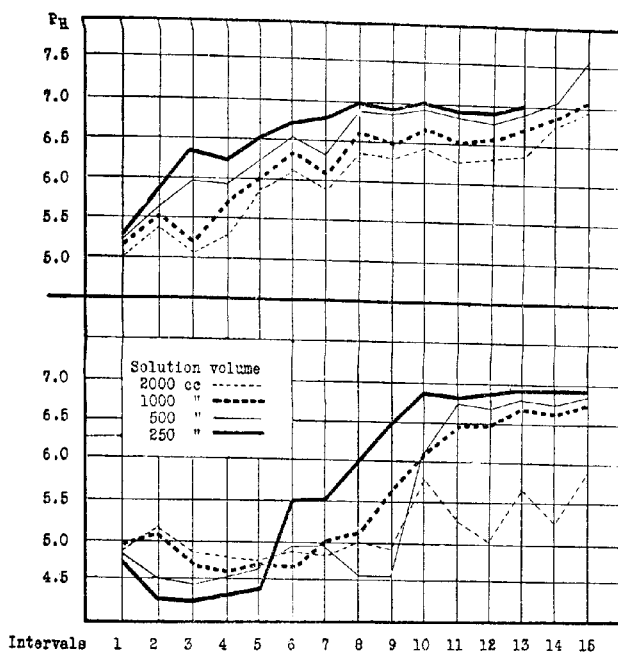


FIG. 3. GRAPHS OF pH VALUES OF CULTURE SOLUTIONS AFTER CONTACT WITH PLANT ROOTS DURING THE VARIOUS GROWTH INTERVALS

Upper graphs, Tottingham's solution  $T_1R_1C_1$ ; lower graphs, modified solution containing ammonium sulfate.

to the fact that in the plants in these cultures chlorosis was delayed although not prevented. Chlorosis invariably occurred in the plants grown in the Tottingham solution following some time after a reaction change which brought the pH values of the solutions above 6.0 regardless of the volume of the solution in which the plants were grown or the amounts of iron added. On the other hand, the plants grown in the 2000-cc. and the 1000-cc. volumes of the solution containing ammonium sulfate were entirely free from chlorosis

appearing, however, near the end of the growth period in the plants grown in the smaller volumes of the solution to which iron was added on the liter basis.

In this series as in the previous ones there is a direct correlation between the reaction of the medium, determined at the end of the growth intervals, and the appearance of chlorosis in the plants grown in each of the different volumes of the Tottingham solution and also in the smaller volumes of the modified Tottingham solution containing ammonium sulfate.

The dry weight data of table 4 again show the retarding influence of the smaller volumes of the culture solutions upon the growth rates of the plants when the solutions are intermittently renewed at intervals of three and one-half days. This is best brought out by the dry weight data obtained from the plants grown in the modified Tottingham solution containing ammonium sulfate since the chlorotic condition of the plants grown in even the smallest volume of this solution was not sufficiently pronounced to become a disturbing factor. The average dry weight per plant of tops and total yields obtained from these cultures vary in the order of the magnitudes of the solution volumes, the lowest average yield per plant in every case being less than half that of the corresponding highest yield. Root yields, on the other hand, do not always vary in the same order as do the top yields nor is the retarding influence of small solution volumes upon the growth of roots so pronounced as it is upon the growth of tops.

#### SUMMARY

The experiments described in this paper were carried out for the purpose of studying the effect of different solution volumes upon the growth of young wheat plants in two types of culture solutions. A study was also made of the hydrogen-ion concentration changes produced by the action of the plants in the different solution volumes and of the influence of these changes upon the availability of iron supplied to the plants in a soluble and an insoluble form.

The types of culture media consisted of the Tottingham solution  $T_1R_1C_3$  at an osmotic concentration value of one atmosphere and this solution modified by substituting ammonium sulfate for the potassium nitrate in equivalent osmotic concentration. The plants were grown in volumes of 250, 500, 1000 and 2000 cc. of each type of solution.

The main results of the experiments may be summarized as follows:

1. Young wheat plants in numbers of not more than three per culture may be grown in the Tottingham solution here used in volumes of not less than 1000 cc. without undue retardation in the rates of growth, if the solutions are renewed at frequent intervals (three days or less) with a suitable form of iron supplied in the proper amounts to prevent chlorosis in the plants. On the other hand, in the modified solution containing ammonium sulfate, the growth rates of the plants varied in the order of the solution volumes from the lowest to the highest, the most vigorous growth by far being produced in the 2000-cc. cultures.

2. Hydrogen-ion concentrations are rapidly decreased by the action of the plants in the Tottingham solution in all stages of development. In the modified solution containing ammonium sulfate the plants increase the hydrogen-ion concentration of the solution slightly during the early stages of growth and decrease it during the later stages. This reversal in the direction of reaction change suggests an important physiological change in the plants with respect to the nitrate and ammonium requirements, involving a change in the absorption rates of the  $\text{NO}_3$ -ions and the  $\text{NH}_4$ -ions.

3. Other things being equal, the rates of reaction change are determined by the volume of the culture solution in which the plants are grown.

4. The availability of iron in the forms here used is determined mainly by the reaction of the culture solution as this is influenced by the action of the plants. A direct correlation is found between the decrease in hydrogen-ion concentration of the culture solutions and the appearance of chlorosis in the plants.

5. Only partial control of the hydrogen-ion concentration of the solutions in contact with the roots of the growing plants may be accomplished by the use of large volumes of solution accompanied by frequent solution renewal.

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## THE TRANSIENT NATURE OF MAGNESIUM-INDUCED TOXICITY AND ITS BEARING UPON LIME-MAGNESIA RATIO STUDIES

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### INTRODUCTORY

In earlier experiments (23) it was found that heavy additions of basic precipitated magnesium carbonate depressed the growth of wheat in baskets. The  $MgCO_3$  treatments at several rates were based upon indications by the Veitch method (33). Those baskets were discarded after the harvesting of the single wheat crop. Consequently, persistence of the initial toxicity was not determined. In a later experiment precipitated magnesium carbonate was added to eight cylinders of a 128-unit series which contained one of the three basket soils. This 128-unit series comprised 4 sub-series, viz., K, 1-32; L, 1-32; M, 1-32; and N, 1-32; of identical treatment but different tillage and cropping. Series K and N were seeded to cowpeas and tall oat grass, respectively, while series L and M were fallow, with and without cultivation. The hydrated magnesium carbonate treatments were equivalent to 8 tons of  $CaO$  per 2,000,000 pounds of soil. After analyses showed that each of the heavy applications of magnesium carbonate had been decomposed (23), all cylinders of series N were seeded to tall oat grass. For three successive seedings the grass died after reaching a height of about three inches (plate 1). A good growth, however, was secured in the equivalent parallels treated with burnt lime, hydrated lime, limestone and dolomite. Differing from the short-period basket studies, the cylinder experiments have been continued over a period of nearly ten years and show the effect of the large magnesium residuals upon later seedings to tall oat grass. Instead of continued toxicity, the effect of the non-carbonate magnesium residuals was soon found to be beneficial. This transition from toxicity to fertility and possible reasons for it are to be considered in this contribution. The bearing of the findings upon the interpretations of earlier studies concerning the hypothesis of an optimum lime-magnesia ratio will also be considered in brief.

In the earlier work, with both baskets and cylinders, only residual carbonates were determined. It was not known whether the depressive influence on the wheat and the tall oat grass was due to an excess of magnesium ions, to an unfavorable hydroxyl-ion concentration, or to an unfavorable balance between calcium and magnesium ions, with or without relationship to the ions of other bases. Whatever the cause of the initial toxicity, it is

certain that the excess of magnesium or the concentration of hydroxyl-ions causing it was derived not from the original precipitated carbonate but from the hydrolysis of the recently precipitated silicate complexes. The increased supplies of magnesium nitrate and magnesium sulfate, resulting from additions of the carbonate, were a factor of possible importance.

Hydrogen electrode studies on the unleached basket soils were conducted by Plummer in 1917. He states (29) that, "The samples which have been treated with excessive amounts of calcium and magnesium carbonates show a greater concentration of OH than H ions in solution. . . . Obviously, the new compounds of calcium and magnesium, whatever they may be, give a strong basic reaction."

#### EXPERIMENTAL

In addition to treatments of precipitated  $MgCO_3$ , the cylinder experiment also called for equivalent additions of  $CaO$ ,  $Ca(OH)_2$ ,  $CaCO_3$ , ground limestone, and ground dolomite. The magnesium carbonate treatments were made only on the 8-ton  $CaO$ -equivalent basis. Comparisons will be made, therefore, with the other materials only as they were chemically equivalent. The same loam soil was used in all 128 cylinders. Description of soil, cylinders, and treatments has been given in a previous contribution (23). The cylinders were placed and treatments made June 11, 1913. Eight weeks later the magnesium carbonate had disappeared and the soil was thus devoid of solid-phase added carbonate after this time (23). Two of the eight magnesium carbonate cylinders, one treated with magnesium carbonate alone and the other with carbonate and manure were seeded to Japan clover, April 7, 1914. Two corresponding cylinders were seeded to tall oat grass on the same date. The remaining four cylinders were left fallow. Because of failure to secure and maintain a stand of Japan clover in the summer, as well as vetch during the winter, especially in the unlimed controls, the clover was later discarded in favor of cowpeas.

#### YIELDS OF TALL OAT GRASS

A good stand of this crop was secured on all of the lime-treated cylinders seeded in April, 1914. However, chlorosis appeared in the growth of the grass on the magnesium carbonate cylinders N-31 and N-32, and gradually every seedling died, apparently as soon as the nutrient of the mother seed had been utilized. These two cylinders were twice re-seeded with the same result. They were then left fallow until October 26, 1914, when seed from an untreated cylinder was used for the fourth seeding. No cuttings of tall-oat grass were made from any of the cylinders during the fall of 1914, but several of the plants of the check cylinder 30, had produced sufficient seed to provide for the re-seeding of no. 31 and 32. The grass was harvested from all the N-series cylinders, those seeded to grass, on June 9, 1915 and a second crop

was harvested in the following fall. These and subsequent yields together with those from chemically equivalent additions of ground limestone and ground dolomite are given in table 1. Growth is shown in plates 2 and 3.

The yields from ground limestone and dolomite are very nearly parallel, both with and without manure. There is no evidence of toxicity from the large amounts of magnesium along with calcium in its native condition.

Comparing cylinders 18 and 32, the precipitated  $MgCO_3$  shows a beneficial effect throughout the first five years, in spite of the delayed benefit. The difference is emphasized during the last two years because of the disappearance

TABLE 1

*Yield of tall-oat grass in cylinders receiving different treatments, all being chemically equivalent to 28,180 lbs. of  $CaCO_3$  per 2,000,000 lbs. of soil*

SERIES NO. CYLINDER NO.	TREATMENT PER 2,000,000 LBS. OF SOIL	AIR DRY WEIGHT									
		1915		1916	1917	1918	1919	1920	1921	Totals	
		1st crop	2nd crop							1915 to 1918	1915 to 1921
		gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
18	30 tons manure	309.4	127.6	67.0	92.5	185.0	81.0	.....	.....	781.5	862.5
21	48 tons manure $CaCO_3$	368.5	158.2	264.0	116.5	207.0	168.0	254.0	345.0	1114.2	1881.2
27	$CaCO_3$	293.7	130.1	232.0	131.8	220.0	186.0	222.0	302.0	1007.6	1717.6
30	Nothing	229.5	117.4	144.0	91.8	196.0	65.0	92.0	34.0	778.6	969.7
31	$MgCO_3$	21.0*	234.7†	22.0	71.5	147.0	70.0	128.0	164.0	496.2	858.2
32	30 tons manure; $MgCO_3$	284.6	193.9	277.0	174.5	249.0	187.0	258.0	307.0	1179.0	1931.0
28	Limestone	308.8	137.8	242.0	141.3	217.0	76.0	259.0	297.0	1046.9	1678.9
29	Dolomite	281.9	122.5	225.0	139.5	265.0	90.0	233.0	242.0	1033.9	1598.9
22	48 tons manure; limestone	367.8	142.9	251.0	107.0	222.0	165.0	273.0	354.0	1090.7	1882.7
23	48 tons manure; dolomite	381.6	141.7	226.0	110.5	167.0	170.0	270.0	355.0	1026.8	1821.8

\* Poor stand. Re-seeded after harvesting first crop.

† Good season after re-seeding.

of the stand in the former cylinder. The yield from the magnesium-carbonate-manure cylinder also is greater than that obtained from the precipitated calcium carbonate cylinders, either alone or when supplemented by manure, for both the initial 5-year period and the full 7-year period.

In cylinder 31, the first crop of 1915 was still subject to the toxic influence of the absorbed magnesium carbonate. It was again seeded, yielding from this second seeding a second crop in 1915 even greater than that produced by its companion cylinder 32, of the carbonate-plus-manure treatment. But a recurrent toxicity again depressed the growth as a result of the direct or indirect influence of the absorbed magnesium carbonate, in the absence of

manure, during 1916 and 1917. In 1917, the depression was less marked and a beneficial influence is shown during the last 3 years, resulting in a marked decrease in the disparity for the 7-year interval, as compared with the initial 5-year period. In fact, the plants which survived after the second crop of 1915 were decidedly more vigorous than those of the control, though fewer in number. The non-carbonate residues from the magnesium carbonate may have produced such changes in the quantity of available nitrate nitrogen and sulfate sulfur as to make them controlling factors in growth after the initial croppings. Occurring after the disappearance of the carbonate, the prevalence of soluble salts and their tendency to leach would cause osmotic changes which might have the effect suggested by Graves and Lund (7). It is evident, therefore, that: (a) Heavy applications of magnesium carbonate, both with and without manure caused a distinct initial toxicity to tall oat grass; (b) the toxic effect was still present at the end of one year, but was not consistently effective 18 months after the addition of carbonate and manure; (c) the toxicity was not due to the presence of magnesium carbonate, for it had disappeared 8 months prior to the first seeding; (d) a beneficial influence appeared in time, as the direct or indirect result of the substances derived from the addition of magnesium carbonate.

#### YIELD OF COWPEAS

The first seeding of cowpeas was made to series K June 16, 1917, or three years after the treatments were applied. Two of these 32 cylinders, received heavy applications of precipitated magnesium carbonate. Table 2 shows that an increased growth of cowpeas, during each of the years 1917, 1918, and 1919 resulted from the additions of the hydrated precipitated carbonate of magnesium. The superiority of the green growth of cylinder 31, which received  $\text{MgCO}_3$  over that of the control was much more striking in appearance than is indicated by the air-dry weight of crops. The cylinder which received precipitated magnesium carbonate and manure apparently produced a much heavier growth than the one which received magnesium carbonate only (plate 4).

The  $\text{MgCO}_3$  treatments, both with and without manure, were responsible for a deep rich green color and an abundance of pods. The  $\text{MgCO}_3$  without manure resulted in a yield more than double that secured from the control and also that from the parallel of calcium carbonate without manure. It also gave greater yields than were obtained from either limestone or dolomite alone, though the reverse was true in the same comparison where manure also was applied. The yield from the 30-ton manure treatment was less than that obtained from  $\text{MgCO}_3$ , or from carbonate and manure. However, the large residue of unfixed precipitated calcium carbonate, with 48 tons of manure, gave a larger yield than the absorbed magnesium carbonate and 30 tons of manure.

It is surprising to note that the total dry weight from cylinder 31,  $\text{MgCO}_3$  alone, was greater than that from cylinder 32, wherein the supplementary treatment of manure was made; for, as is indicated by plate 4, the growth of stems, leaves and pods in the latter instance appeared much heavier. The manure apparently induced a much greater yield, in harmony with the yields from precipitated carbonate, limestone and dolomite alone, as compared with the respective treatments carrying manure. In spite of the appearance to the contrary, however, the data of each of the years 1917, 1918, and 1919 show a greater yield for the  $\text{MgCO}_3$  alone, than for the carbonate plus manure.

If the amount of magnesium extracted from the non-carbonate residuals in the soil were the primary factor in causing toxicity, the manure additions would be expected to have intensified the toxic effect by increasing the amount

TABLE 2  
*Yield of cowpea hay in cylinders variously treated, all treatments being chemically equivalent to 28180 lbs. of  $\text{CaCO}_3$  per 2,000,000 lbs. of soil*

SERIES & CYLINDER NO.	TREATMENT PER 2,000,000 LBS. OF SOIL	YIELD OF HAY, AIR-DRIED			
		1917	1918	1919	Total
		gms.	gms.	gms.	gms.
18	30 tons manure	309.3	299.5	134.5	743.3
21	48 tons manure; $\text{CaCO}_3$	310.8	442.3	240.5	993.6
27	$\text{CaCO}_3$	218.8	40.5	83.5	342.8
30	Nothing	164.3	221.8	34.5	420.6
31	$\text{MgCO}_3$	261.8	307.0	302.0	870.8
32	30 tons manure; $\text{MgCO}_3$	250.0	260.5	291.0	801.5
28	Limestone	161.0	280.3	153.5	594.8
29	Dolomite	139.8	192.3	266.5	598.6
22	48 tons manure; limestone	356.0	472.0	344.0	1172.0
23	48 tons manure; dolomite	388.3	401.0	461.8	1251.1

of magnesium available to the plant, unless a corrective balance were maintained by parallel increases in the amounts of the other nutrients supplied either directly or indirectly by the manure. For, the free water of the soil would have become richer in  $\text{CO}_2$  derived from the added organic matter, and being a stronger solvent it would have caused a more extensive hydrolysis of the non-carbonate magnesium residuals.

In agreement with the tall-oat grass yields, the cowpeas from the dolomite treatments, with and without manure, fail to show any toxic effect of the magnesium of the dolomite, nor any inferiority to those of the limestone treatments.

Since it was not known, in the case of the cowpeas, whether an initial and transient toxicity would have preceded the fertility of the later period, as in the case of the tall-oat grass, information on this point was sought.

INITIAL TOXICITY OF  $MgCO_3$  ON COWPEAS IN WAGNER POTS

The same soil used in the cylinders was taken for a series of pots in experiments to determine any initial toxicity of magnesium toward cowpeas. However, the plot from which the pot soil was taken had been lightly limed twice previously within a period of 14 years, and small specks of unabsorbed calcium carbonate were still visible. Four Wagner pots were used, each containing 7,150 gm. of air-dry soil. To each of three of these was added 99,3405 gm. of analyzed precipitated  $MgCO_3$ , equivalent to 28,180 pounds of  $CaCO_3$  per 2,000,000 pounds of soil. The  $MgCO_3$ , used was a composite of several samples obtained from the California, New York, Mississippi and Missouri Experiment Stations. The control pot received no  $MgCO_3$ . In order to insure against potash deficiency, each pot received 0.4250 gm. of KCl. The treatments were added August 2, 1918. The following day, the soil was brought to a moisture content of 30 per cent, on the air-dry basis and the cowpeas were sown to the control and to one treated pot. The moisture content was then permitted to drop to 20 per cent, and there maintained during the experiment. The original air-dried soil contained 0.072 per cent  $CO_2$  which was increased to 0.415 per cent after the addition of hydrated magnesium carbonate. Subsequent analyses of sun-dried freshly sampled soil showed a  $CO_2$  content of 0.259 per cent on August 14, 0.261 per cent on August 29, and 0.255 per cent on September 24, indicating a distinct retardation of the initial rapid rate of  $MgCO_3$  absorption, as influenced by the two previous additions of burnt lime.

Eight seeds were sown in the control and in one  $MgCO_3$ -treated pot. However, since all but two of the seeds died in the treated pot shortly after sprouting, the plants in the control were thinned to the same number. By September 6 chlorosis was so severe that it became necessary to harvest the plants in order to secure satisfactory photographs (plate 5, fig. A). The second seeding was made to another treated pot and control September 24, 1918; the plants were thinned as formerly and the crops were harvested and photographed October 23. As indicated by plate 5, fig. B, the initial toxicity was still pronounced. The control and the third magnesium-treated pot were then kept moist without leaching, until September 3, 1919, thirteen months after the treatment, when they were seeded to cowpeas from the same lot of seed previously used. Thirty days later the plants, thinned to five, were harvested and photographed with the result shown in plate 5, fig. C. The green weight of the plants from the control was 19.07 gm. as against 24.40 gm. for those in the treated pot. The color of the plants grown in the soil treated with magnesium carbonate was much deeper than that of the control plants. It is apparent, therefore, that without leaching the aging of the magnesium additions in moist soil greatly lessened the initial toxicity, if it did not convert it to fertility. From the growth in the Wagner pots it seems reasonable to conclude that the magnesium carbonate additions in the cylinders would have been toxic to the cowpeas as in the case of the tall oat grass, had the cowpeas and grass been sown at the same time.

THE ACCUMULATION OF WATER-SOLUBLE SALTS OF CALCIUM AND MAGNESIUM  
IN THE WAGNER POTS

Lysimeter investigations upon the soil used in both cylinders and Wagner pots demonstrated that the heavy treatment of precipitated  $\text{MgCO}_3$  had caused an extensive loss of both nitrates and sulfates during the first year after treatment. It appeared then that the cowpea growth in the Wagner pots took place in spite of the presence of a large amount of these two soluble salts of magnesium. The lysimeter results also indicated that the maximum production of the magnesium salts had taken place prior to the final tall oat grass seeding, which was a year after treatment. But, notwithstanding the leaching from the cylinders during the first 12 months after treatment, and the absence of any residual carbonate during the latter 10 months of the period, the first three seedings of tall oat grass had been killed off.

TABLE 3  
*Soluble calcium in soil from two Wagner pots at time of harvesting cowpeas*

ZONE	TREATMENT	IN 120 GM. OF AIR-DRY SOIL			CaSO <sub>4</sub> PER 2,000,000 LBS. AIR-DRY SOIL, IF ALL Ca WERE PRESENT AS CaSO <sub>4</sub>
		Determined as CaO	CaSO <sub>4</sub> —if present as sulfate		
		gm.	gm.	percent	
Upper	None	0.0155	0.0376	0.0321	642
	MgCO <sub>3</sub>	0.0067	0.0163	0.0139	278
Middle	None	0.0157	0.0381	0.0324	648
	MgCO <sub>3</sub>	0.0057	0.0138	0.0118	236
Lower	None	0.0147	0.0357	0.0304	608
	MgCO <sub>3</sub>	0.0049	0.0119	0.0101	202
Average	None	0.0153	0.0371	0.0316	632
	MgCO <sub>3</sub>	0.0058	0.0141	0.0119	238

The soil of the unleached Wagner pots, used in the observations of initial toxicity, was subjected to water extraction analysis to determine soluble calcium and magnesium salts present at the period when toxicity was no longer evidenced by cowpeas. The soil was removed in three 2½ inch layers, which will be spoken of as the upper, middle, and lower zones; 750 gm. of soil from each zone was mixed with 3,500 cc. of distilled water, the suspensions agitated for 8 hours, permitted to stand overnight and filtered. Three liters of the filtrate were concentrated and aliquots used in determinations of calcium, magnesium and sulfates. The results given in tables 3, 4 and 5, and titrations show that the recovered bases were present as neutral calcium and magnesium salts engendered and accumulated over the 14 month interval of moist contact, rather than the relatively insoluble carbonate forms. The



extraction aliquots from 120 gm. fractions gave recoveries of 0.0155 gm., 0.0157 gm. and 0.0147 gm. of CaO, table 3, from the upper, middle and lower zones of the control pot represent respectively 642 lbs., 648 lbs. and 608 lbs., per 2,000,000 lbs. of air-dry soil, if the dissolved calcium was present as

TABLE 4  
*Soluble magnesium in soil from two Wagner pots at time of harvesting cowpeas*

ZONE	TREATMENT	IN 120 GM. OF AIR-DRY SOIL			MgSO <sub>4</sub> PER 2,000,000 LBS. AIR-DRY SOIL, IF ALL Mg WERE PRESENT AS MgSO <sub>4</sub>
		Determined as Mg <sub>2</sub> P <sub>2</sub> O <sub>7</sub>	MgSO <sub>4</sub> —if present as sulfate		
		gm.	gm.	per cent	lbs.
Upper	None	0.0078	0.0084	0.0072	144
	MgCO <sub>3</sub>	0.1104	0.1194	0.1078	2156
Middle	None	0.0067	0.0072	0.0061	122
	MgCO <sub>3</sub>	0.1224	0.1324	0.1129	2258
Lower	None	0.0060	0.0065	0.0055	110
	MgCO <sub>3</sub>	0.1119	0.1210	0.1031	2062
Average	None	0.0068	0.0074	0.0063	126
	MgCO <sub>3</sub>	0.1149	0.1243	0.1079	2158

TABLE 5  
*Soluble sulfates in soil from Wagner pots at the time of harvesting cowpeas*

ZONE	TREATMENT	IN 120 GM. AIR-DRY SOIL						SULFATES IN 2,000,000 LBS. AIR- DRY SOIL	
		BaSO <sub>4</sub> deter- mined	CaSO <sub>4</sub> equivalent			MgSO <sub>4</sub> equivalent		If present as CaSO <sub>4</sub>	If present as MgSO <sub>4</sub>
			gm.	gm.	per cent	gm.	per cent		
Upper	None	0.0486	0.0283	0.0241	0.0251	0.0213	482	426	
	MgCO <sub>3</sub>	0.0646	0.0377	0.0321	0.0333	0.0283	642	566	
Middle	None	0.0355	0.0207	0.0177	0.0183	0.0156	354	312	
	MgCO <sub>3</sub>	0.0386	0.0225	0.0192	0.0199	0.0190	384	380	
Lower	None	0.0321	0.0187	0.0159	0.0166	0.0142	318	284	
	MgCO <sub>3</sub>	0.0379	0.0221	0.0188	0.0195	0.0166	376	332	
Average	None	0.0387	0.0226	0.0193	0.0200	0.0170	386	340	
	MgCO <sub>3</sub>	0.0470	0.0274	0.0233	0.0242	0.0206	466	412	

calcium sulfate. These calcium recoveries and the much smaller amounts of soluble magnesium recovered from the control demonstrate that nitrate and sulfate generations were cared for mainly by native calcium rather than native magnesium. On the other hand, the calcium recoveries from the

magnesium treatment were decidedly less than those from the control. This shows that nitrates and sulfates engendered in the magnesium-treated pot were neutralized by the absorbed magnesia, which in this way exerted a protective action upon the native soil calcium.

The large gains in soluble magnesium recovered from the magnesium treatment in all zones show that more than 2000 pounds of magnesium sulfate equivalent would have been accounted for upon the assumption that this salt was leached. However, the sulfate determinations and hypothetical combinations of table 5, and the fact that only small amounts of chlorine were leached made it necessary to conclude that most of the magnesium losses were due to extractions of magnesium nitrate. This agrees with unpublished lysimeter data upon the same soil and treatment which showed the hydrated magnesium carbonate to be very active in increasing leachings of nitrates.

If all of the sulfate radical were combined with calcium, there would be no great excess of calcium sulfate and the differences between the amounts in the several zones would not be great; nor would the average amount of the control differ greatly from that of the magnesium carbonate treatment, 386 lbs. as against 466 lbs. The same statements hold in the case of the assumption that all soluble  $\text{SO}_4$  was combined with magnesium, the averages of the control and the magnesium-addition pots being 340 lbs. and 412 lbs., respectively, per 2,000,000 lbs. of soil. However, in spite of the appreciable quantities of sulfates and the larger quantities of magnesium nitrate, the cowpeas were growing at the time of harvest and soil analysis, without any apparent indication of the toxicity, which was so marked in the two first plantings. The amounts of soluble salts formed in the unleached pots could hardly be as great during the first 30 days after treatment, nor during the first eleven weeks, as were the amounts which accumulated during the longer period of 14 months. Yet, toxicity was manifested in the case of the peas growing during the first 30 days and in the case of those grown during a 30-day period which began 51 days after the incorporation of the magnesium carbonate, though it was not indicated in the 30-day period between the thirteenth and fourteenth months after treatments, at which time the excessive accumulations of neutral salt were found by the water extractions.

Tolerance to the magnesium ion could not have been established under the conditions of the pot experiments and yet, coincidently with probably greater soluble-salt accumulation over that present initially, the toxic influence apparently had been dissipated. This would indicate rather strongly that a change in reaction had taken place. Thus, initially, there probably occurred a smaller accumulation of neutral and soluble magnesium salts and a larger hydroxyl-ion concentration; and later, a larger occurrence of the neutral magnesium salts, accompanied by a decrease in hydroxyl-ion concentration. An accumulation of the neutral salts of the magnesium would tend to depress, or force back, the dissociation of the basic salts whereby an excess of hydroxyl-ions would less readily occur. While this might account for the

dissipation of the toxicity in the case of the unleached Wagner pots, it would hardly apply in the case of the field cylinders where leaching was in effect. It may be that the hydroxyl-ion concentration was reduced in the cylinders after the early disappearance of the more readily dissolved residual carbonate, together with the retardation of hydrolysis, because of the applied magnesium becoming more firmly absorbed and fixed. This question was not involved in the result sought in the original planning of the cylinder experiments; how-

TABLE 6  
*pH values of distilled water extracts of cylinder soils previously treated with precipitated carbonates of calcium and magnesium—extractions obtained by agitation and over night contact. Ratio of soil to water was 1 to 25*

TREATMENT APPLIED JUNE, 1913*	DATE OF SAMPLING AND BRINGING TO AIR-DRY CONDITION	INDICATORS USED AND DETERMINATIONS OBTAINED MARCH 10, 1922				
		Methyl red, range 4-6.0	Brom-cresol purple, range 5.2-7.0	Brom-thymol blue, range 6.0-7.8	Phenol red, range 6.8-8.6	Cresol red, range 7.2-9.0
None†	May 6, 1918	→	5.9	←	←	←
None‡	June 18, 1918	5.2	5.6	←	←	←
CaCO <sub>3</sub> †	June 18, 1918	→	6.5	6.8	7.1	←
MgCO <sub>3</sub> †	Aug. 15, 1913	→	6.0	6.2	←	←
MgCO <sub>3</sub> ‡	May 6, 1918	5.9	5.6	←	←	←

\* All additions equivalent to 28180 lbs. CaCO<sub>3</sub> per 2,000,000 lbs. of soil.

† Composite of 4 cylinders.

‡ Composite of 8 cylinders.

TABLE 7  
*pH values of carbonated water extracts of cylinder soils, previously treated separately with precipitated carbonates of calcium and magnesium—extractions obtained by agitation and overnight contact. Ratio of soil to carbonated water 1 to 25.*

TREATMENT APPLIED JUNE, 1913*	DATE OF SAMPLING AND BRINGING TO AIR-DRY CONDITION	INDICATORS USED AND DETERMINATIONS OBTAINED MARCH 10, 1922			
		Brom-phenol blue, range 3.4-4.8	Methyl-red, range 4.4-6.0	Brom-cresol purple, range 5.2-7.0	Brom-thymol blue, range 6.0-7.8
None†	May 6, 1918	→	5.1	←	←
None‡	June 18, 1918	→	5.0	←	←
CaCO <sub>3</sub> †	June 18, 1918	→	5.5	←	←
MgCO <sub>3</sub> †	Aug. 15, 1913	→	5.6	←	←
MgCO <sub>3</sub> ‡	May 6, 1918	→	5.4	←	←

\* All additions equivalent to 28180 lbs. CaCO<sub>3</sub> per 2,000,000 lbs. of soil.

† Composite of 4 cylinders.

‡ Composite of 8 cylinders.

ever, it was thought well to determine the pH values of solutions of the composite samples used also for the analytical work reported in tables 10, 11, and 12. These values are given in tables 6 and 7.

The laboratory samples taken in the early part of the initial year and at the close of the five-year period were used. They had been preserved in air-

dry condition. The extract were made with both distilled and carbonated water, using 1 gm. soil to 25 cc. water with overnight contact. The method used was that of Van Alstine (32).

*Distilled water extracts:* The distilled water extracts give pH values in each case below those of neutrality. The highest value is that derived from the calcium carbonate treatment and in this instance an excess of carbonate is present in the soil. The pH value of the magnesium-treated carbonate-free sample of August 15, 1913, taken 8 weeks after treatment is higher than that of the same soil sampled May 6, 1918. The soil, which remained subject to both leaching and continued dynamic influences while moist, is therefore of lower pH value than the soil which was more quickly brought to the air-dry state and kept in that condition.

*Carbonated water extracts:* The carbonated water extracts show a less pH value in each case than was shown by the distilled water extracts. A slight difference is shown between the values given to the two samplings of the magnesium carbonate additions, the first of which is slightly nearer, and the second slightly farther from neutrality than the reading secured from the calcium carbonate treatment which still carries a carbonate excess, as contrasted to the absence of residual carbonate in the case of the magnesium carbonate additions. Toxicity may have been induced, therefore, by readily soluble magnesium complexes shortly after their fixation, a progressive change in pH values following parallel with the aging of the fixed base.

#### POSSIBLE REASONS FOR THE INITIAL TOXICITY AND ITS SUBSEQUENT DISAPPEARANCE

The age of a precipitate is one factor which influences its solubility coefficient. The carbonate-silicate study of MacIntire and Willis (22) has shown that an excess of magnesium carried by an aged and complex silicate, serpentine, was not toxic when applied in heavy amounts to the Cumberland loam used in the cylinders and lysimeters. We have shown also (19, 23) that the silicate reaction may be reversed with an appreciable quantity of magnesium entering into the carbonated water solution, which bathes the mass of soil containing freshly precipitated siliceous complexes. The finely ground natural serpentine, however, is but slightly acted upon by the same agency under similar conditions. Since it was known that the magnesium carbonate had become fixed, or silicated, prior to the tall oat grass seeding, as established by carbonate determinations, and that the greater part of the applied magnesium was still present in the soil, as indicated by data obtained from analyses of lysimeter leachings, it seemed logical to assume that the newly formed siliceous compounds had progressively passed into more complex and more stable complexes. Such an hypothesis would suggest that the fixed magnesium would have a decreasing tendency to hydrolyze. Were the basic components of the freshly formed magnesium complexes to undergo progressive decrease in their total amounts through leaching, while remaining of constant proportions, they could be

reasonably expected to decrease in solubility, or tendency to undergo hydrolysis and re-carbonation in the free carbonated water of the soil. It is possible, however, under the dynamic conditions inherent to the soil mass, that the several basic elements leached out in different proportions and that successive progressive complexities and changes in proportion of bases would ensue, thereby converting the added magnesium into combinations more firmly fixed.

Thus, applying the related lysimeter and other studies to the cylinder results, there was probably an abundance of magnesium forced upon the growing plants in the cylinders, not only as magnesium bicarbonate, but also as the biological end-products, nitrate and sulfate, as a result of accelerated nitrification and sulfonation induced by the bicarbonate derived from the ready hydrolysis of the freshly precipitated silicates. Although Kelly (9) showed, in incubation studies, that basic magnesium carbonate caused a distinct retarding of nitrification of added nitrogenous materials, we have abundant data to the opposite effect, in so far as native nitrogenous substances are concerned. Sulfonation, as measured by accelerated outgo of sulfates, was also found to have been greatly increased by magnesium additions to the same loam in the lysimeters.

With the aging process continued, and after passage of the more readily soluble salts down into the subsoil, the soil medium apparently approached the normal, with a diminution of the intense initial toxicity. Such an explanation would explain in a measure the initial phenomenon, whether the cause be attributed to an excess of magnesium ions or to an excessive hydroxyl-ion concentration.

An accumulation of magnesian salts may have caused changes in osmotic pressure, particularly during the earlier periods. Ewart (3) studied the influence of high salt concentrations in the soil solution and found a distinct retarding in germination of seed. While a splendid germination was secured from our seeding of tall oat grass, it is quite possible that osmotic pressure had some effect upon growth.

Viewed from another angle, the toxicity might be considered as the result of disrupted equilibria, if extensive basic interchange were assumed. Assuming that magnesium carbonate liberated and caused excessive leaching of calcium, sodium, and potassium from their native combinations, such basic replacement during the period of greatest transformation of magnesium carbonate into non-carbonate forms would tend toward beneficial antagonisms rather than an excess of magnesium and a paucity of calcium and the alkalis. We have data in manuscript form which bears directly upon such an assumption for this particular soil. But, the toxicity noted prevailed much longer. Furthermore, the writer (20) has shown that if sodium and potassium are liberated by calcium and magnesium in this loam, the liberated alkalis do not pass into the free water of the leachings. This finding is in harmony with that of Lyon and Bizzell (17, 18). Nevertheless, enough soluble magnesium salts would still be available to maintain a high ratio of magnesium to calcium. This would tend

to maintain toxicity unless the plant were to become magnesium tolerant. But, instead of initial fertility and subsequent toxicity the reverse order was definitely established for tall oat grass and also, later, for cowpeas. The temporary toxicity to tall oat grass and the subsequent fertility when leaching was permitted under normal field conditions, the same tendency later in the basket work with cowpeas where no leaching took place, the fact that toxicity ceased although most of the added magnesium was still in the soil and with pH values undetermined seem to justify the conclusion that the residual magnesian materials had undergone some decided change in parallel to the occurrence of initial toxicity and subsequent fertility.

#### LOSSES OF MAGNESIUM DURING THE 5-YEAR PERIOD

One year after the beginning of the cylinder studies, a 46-unit lysimeter equipment was installed, the same loam being used in both cylinders and lysimeters. All of the basic materials used in the cylinders were also used in the lysimeters in amounts equivalent to 8 tons of CaO per 2,000,000 pounds of soil. The only difference between the methods of incorporating materials was that the materials were mixed throughout the upper 6 of the 8 inches of soil in the cylinders, while lysimeter treatments were mixed throughout the full 8-inch depth. The losses from the cylinders from 1913-1917 are taken as comparable to those from the lysimeters during 1914-1918.

The data of table 8 also show the amounts of surface soil leachings of magnesium and calcium salts stopped by the subsoil. The heavy loss of 1914-1915, equivalent to 42 per cent of the 8-year total resulted from an annual rainfall of 47.96 inches. Since a large part of the magnesium added was still present in the soil in 1919, the decreased leaching indicates that the portion still held by the soil had decreased in solubility. Comparing tank 4 with tank 25, it appears that the foot of subsoil stopped, in round numbers, 3,300 lbs., 1,400 lbs., 1,100 lbs., 400 lbs. and 500 lbs. for the first five years, respectively. The same order of differences will be noted for the MgO-treatments. Each annual surface soil outgo of magnesium from the  $MgCO_3$  treatment was respectively greater than the amount of calcium lost by the corresponding calcium treatment. The same relation also holds true in a similar comparison between MgO and CaO, in the surface soil tanks. This obtains, although the disparity between the respective solubilities of precipitated magnesium carbonate and calcium carbonate in carbonated water was offset by the far greater speed in the absorption, or fixation, of the magnesium carbonate by the soil.

A previous contribution (23) and table 10 show that the solubility of the residual calcium carbonate from a heavy treatment is greater than that of the residual absorbed non-carbonate magnesian compounds, when the treated soil is extracted with carbonated water. But, it is quite possible that another factor may be involved where the  $CO_2$  of the soil water is derived from that generated within the soil. Since combinations of magnesium were more active

TABLE 8  
*Amounts of  $MgCO_3$  and  $CaCO_3$  leached from 8-ton treatments of 93.0 per cent  $CaO$ , and equivalents of  $MgO$ ,  $CaCO_3$  and  $MgCO_3$  per 2,000,000 lbs. of soil in terms of  $CaCO_3$  equivalence as pounds per acre. 1914-1919*

TANK NUM- BER	TREAT- MENT	AS TO SUBSOIL	1914-1915			1915-1916			1916-1917			1917-1918			1918-1919			TOTAL 1914-1919		
			$CaCO_3$ lbs.	$MgCO_3$ lbs.	Total lbs.	$CaCO_3$ lbs.	$MgCO_3$ lbs.	Total lbs.	$CaCO_3$ lbs.	$MgCO_3$ lbs.	Total lbs.	$CaCO_3$ lbs.	$MgCO_3$ lbs.	Total lbs.	$CaCO_3$ lbs.	$MgCO_3$ lbs.	Total lbs.	$CaCO_3$ lbs.	$MgCO_3$ lbs.	Total lbs.
1	$CaO$	None	1767	112	1879	1062	61	1123	1009	36	1045	446	39	485	732	44	776	5016	292	5308
22	$CaO$	1 foot	499	279	778	408	300	708	410	323	733	197	165	362	305	260	565	1819	1327	3146
2	$MgO$	None	180	2274	2454	74	1346	1420	164	1448	1612	61	570	631	123	817	940	602	6455	7057
23	$MgO$	1 foot	439	239	678	477	361	838	442	374	816	195	252	447	306	240	546	1859	1466	3325
3	$CaCO_3$	None	1406	149	1555	831	58	889	913	74	987	453	48	501	749	48	797	4352	377	4729
24	$CaCO_3$	1 foot	350	184	534	350	250	600	435	356	791	174	205	379	383	301	684	1692	1296	2988
4	$MgCO_3$	Note	189	3525	3714	79	1791	1870	145	1569	1714	56	631	687	113	787	900	582	8303	8885
25	$MgCO_3$	1 foot	388	210	598	470	338	808	551	480	1031	180	231	411	293	280	573	1882	1539	3421

than those of calcium upon the soil flora, as determined by the leached nitrate and sulfate end-products, it is quite possible that compounds of magnesium may also cause a greater generation of  $\text{CO}_2$  from soil organic matter and produce a more strongly carbonated soil-water with a greater solvent action than that induced by calcareous materials. Again, it is quite possible that with equal generation of  $\text{CO}_2$  the magnesian compounds may materially depress a soil's retentive properties for  $\text{CO}_2$  causing it to yield that gas more readily to the free water of the soil.

As applied to the cylinder studies upon plant growth, these observations strengthen the hypothesis that the disappearance of the toxicity induced by the non-carbonate residuals derived from the applied magnesium was not caused so much by its elimination through leaching, as by the fact that the residues in the cylinders were not so active and therefore possessed of less tendency to produce those conditions which proved toxic to plant growth. The data do not determine, however, whether the apparently diminished activity and the disappearance of toxicity were caused by a lessened production of magnesium nitrate, or magnesium sulfate—excesses of magnesium—ions—or to a diminished hydroxyl-ion concentration, but the probability of the latter was indicated by the pH values given in tables 6 and 7.

CONCERNING COMBINATIONS IN WHICH SOLUBLE MAGNESIUM MAY HAVE  
LEACHED FROM THE CYLINDERS

While the data of table 8 set forth the total amounts of calcium and magnesium leached from the magnesium carbonate and equivalent magnesium oxide treatments, as well as the same losses from parallel and equivalent treatments of both carbonate and oxide of calcium, they do not specifically determine the combinations carried in the drainage waters. This information as to magnesium calcium outgo is of value in considering reasons for the occurrence and subsequent disappearance of the toxicity found in the nearly parallel cylinder experiments. MacIntire, Willis and Holding (24) and subsequent unpublished data have shown the tendencies of the oxides and carbonates of calcium and magnesium to materially alter the normal oxidative processes responsible for the generation of leached sulfates. Table 9 shows the ratio of the total nitrates and sulfates to the amount of bicarbonates leached. The totals of magnesium and calcium carried through as neutral salts, expressed as calcium-carbonate-equivalence, are used in comparisons with the total magnesium and calcium bicarbonates, determined by titration and expressed in terms of calcium carbonate. Direct determinations of potassium in the leachings for the initial 5-year period previously reported by the senior author (20) together with differential determinations of concurrent sodium have established the fact that these alkalies did not come out in large enough quantities to alter the general relations. Table 9 shows that the clay subsoil consistently stopped both neutral salts and bicarbonates of both calcium and magnesium. It may be assumed that the upper part of the subsoil became



TABLE 9  
Amounts of magnesium and calcium salts from 8-ton equivalent treatments of 93 per cent CaO, MgO, CaCO<sub>3</sub> and MgCO<sub>3</sub>, per 2,000,000 lbs. of soil in terms of CaCO<sub>3</sub> equivalence as lbs. per acre—Annual periods 1914-1919

TANK NUM- BER	TREAT- MENT	AS TO SUBSOIL	1914-1915				1915-1916				1916-1917				1917-1918				1918-1919				TOTALS 1914-1919			
			Calcium and magnesium bicarbonate		Total calcic and mag- nesic salts as CaCO <sub>3</sub>		Calcium and magnesium bicarbonate		Total calcic and mag- nesic salts as CaCO <sub>3</sub>		Calcium and magnesium bicarbonate		Total calcic and mag- nesic salts as CaCO <sub>3</sub>		Calcium and magnesium bicarbonate		Total calcic and mag- nesic salts as CaCO <sub>3</sub>		Calcium and magnesium bicarbonate		Total calcic and mag- nesic salts as CaCO <sub>3</sub>		Calcium and magnesium bicarbonate		Total calcic and mag- nesic salts as CaCO <sub>3</sub>	
			lbs.	per cent	lbs.	per cent	lbs.	per cent	lbs.	per cent	lbs.	per cent	lbs.	per cent	lbs.	per cent	lbs.	per cent	lbs.	per cent	lbs.	per cent	lbs.	per cent	lbs.	per cent
1	CaO	None	1879	494.9	26.3	1123	665.8	59.3	1045	750.6	71.8	485	330.7	68.2	776	449.7	57.5	5308	2691.7	50.7	4368.2	61.9				
22	CaO	1 foot	778	112.4	14.4	708	176.4	24.9	733	203.9	27.8	362	148.8	41.1	565	154.3	27.8	3146	795.8	25.3	886.2	26.7				
2	MgO	None	2454	1128.7	46.0	1420	1032.8	72.7	1612	1150.7	71.4	631	469.6	74.3	940	586.4	62.4	7057	4368.2	61.9						
23	MgO	1 foot	678	108.0	15.9	838	168.6	20.1	816	231.5	28.4	447	197.3	44.1	546	180.8	33.1	3325	886.2	26.7						
3	CaCO <sub>3</sub>	None	1555	181.9	11.7	889	478.4	53.8	987	622.8	63.1	501	316.3	63.1	797	476.2	59.8	4729	2075.6	43.9						
24	CaCO <sub>3</sub>	1 foot	534	115.7	21.7	600	154.3	25.7	791	217.1	27.4	379*	43.0*	11.3	684	187.4	27.4	2988	717.5	24.0						
4	MgCO <sub>3</sub>	None	3714	2432.7	65.5	1870	1494.7	79.9	1714	1369.0	79.9	687	504.8	73.5	900	563.2	62.6	8885	6364.4	71.6						
25	MgCO <sub>3</sub>	1 foot	598	108.0	18.0	808	179.7	22.2	1031	249.1	24.2	411	164.2	40.0	573	181.9	33.5	3421	882.9	25.8						

\* Outlet became plugged which produced waterlogged condition and reduced number of leachings until trouble was rectified.

saturated in effecting this absorption phenomenon and that the plant roots had access to the calcium and magnesium held in this way. Except for this point, the data relating to the tanks with subsoil will not be considered further. The  $\text{CaO}$  and  $\text{CaCO}_3$  additions did not prove toxic when applied in nearly equal amounts in the cylinders and are included only because of the comparative relationships. The data on the chemically equivalent application of magnesium oxide are of value because of relationship to the carbonate and for some references yet to be made.

Bicarbonates, known by analysis to be principally magnesium bicarbonate, leached from cylinders treated with magnesium oxide and carbonate amounted, respectively, to 46.0 per cent and 65.5 per cent of the total alkali-earth losses during the initial year. These magnesium bicarbonate losses from the magnesian additions are materially greater than those of calcium bicarbonate from the corresponding treatments of calcium oxide and carbonate. The total outgo of alkali-earths decreased after the initial year. The same is true of both neutral salts and bicarbonates; but, the greater relative decrease in the former causes a general increase in the percentage relationship of bicarbonates to the total, this being true for both calcium and magnesium additions. However, table 13 shows that the increasing percentage of bicarbonates to the total is derived mainly from residual calcium carbonate in the case of the calcium treatment; while a lesser carbonate residual in the case of additions of magnesium oxide and carbonate means a greater supply of magnesium derived through the hydrolysis of the absorbed non-carbonate residuals from the added magnesian materials. In spite of the large residual stores of magnesian materials, it is evident that the amounts of magnesium carried by the leachings are materially decreased after the first year. In the case of the  $\text{MgO}$  treatment, it appears that the decrease is mainly due to a lessened production of neutral salts during the second and third year more particularly, since the absolute amount of bicarbonates yielded each year of this two-year period closely approach the outgo during the initial annual period. However, in the case of the magnesium carbonate treatment, both neutral magnesium salts and bicarbonates suffer a marked decrease, the ratio of bicarbonate to total magnesium salts increasing somewhat during both the second and third yearly periods. The decrease during the fourth and fifth annual periods is still more marked, notwithstanding the magnitude of the differentials between the applied materials and the recoveries. Such differentials represent well disseminated residuals which would be expected to yield quantities ample to effect an approximation to the concentrations maintained by the earlier leachings, if the remaining stores were readily soluble. It would thus appear that the large amount of magnesium remaining had become less soluble. For the 5-year period, 1914-1919, comparable to the 5-year period, 1913-1918, in the cylinder experiments, the total magnesium outgo from the  $\text{MgO}$  treatment was equivalent to 7057 pounds of calcium carbonate. Of this amount an outgo of 4368 pounds, or 61.9 per cent, is accounted for by the bicarbonates of the

alkali-earths, as against 2689 pounds, or 38.1 per cent, for the neutral salts of the same elements. In the case of the corresponding  $\text{MgCO}_3$  addition, the total magnesium outgo equivalent to 8885 pounds of  $\text{CaCO}_3$  is accounted for by assigning 6362 pounds, or 71.6 per cent, to the bicarbonate form and 2523 pounds, or 28.4 per cent, to the neutral salts.

SOLUBILITY OF THE FIXED MAGNESIUM DERIVED FROM THE MAGNESIUM CARBONATE TREATMENTS—CYLINDER EXPERIMENTS

The ready solubility of the large non-carbonate magnesium residual derived from the applied carbonate shortly after its incorporation may be reasonably assigned as the cause of the initial toxicity. A diminished solubility 5 years

TABLE 10

*Calcium and magnesium dissolved by a 4-hour agitation and extraction with carbonated water from 25-gm. charges of composite samples from rims treated separately with precipitated  $\text{CaCO}_3$  and precipitated  $\text{MgCO}_3$ , June, 1913*

TREATMENT		DATE OF SAMPLING	TOTAL CHEMICAL EQUIVALENCE OF TITRATIONS OF CARBONATED WATER EXTRACT		CALCIUM		MAGNESIUM	
Material applied to soil	CaCO <sub>3</sub> or $\approx$ per 2,000,000 lbs. of soil		As CaCO <sub>3</sub> $\approx$	As CaCO <sub>3</sub> per 2,000,000 lbs. of soil	As CaCO <sub>3</sub> per 25 gm. of soil	As CaCO <sub>3</sub> per 2,000,000 lbs. of soil	As CaCO <sub>3</sub> per 25 gm. of soil	As CaCO <sub>3</sub> per 2,000,000 lbs. of soil
			gm.	lbs.	gm.	lbs.	gm.	lbs.
None*	lbs.							
		May 6, 1918	0.0250	2000	0.0080	640	0.0128	1024
MgCO <sub>3</sub> †	28180	Aug. 15, 1913	0.0535	4280	0.0083	664	0.0580	4640
MgCO <sub>3</sub> †	28180	May 6, 1918	0.0380	3040	0.0066	528	0.0300	2400
CaCO <sub>3</sub> †	28180	June 18, 1918	0.2420	19360	0.2355	18840	0.0082	656

\* Composite of 4 cylinders.

† Composite of 8 cylinders.

later may be attributed to decreased solubility of the residual, or a marked decrease in the amount of the residual. Were the magnesium combinations of the soil of the same chemical composition at the time of the first and fifth year's samplings, the comparative solubilities would be determined by the total amounts remaining at the two dates and upon variation in the tenacity of the fixation, or complexity, as influenced by the time factor, that is, the aging process. Data upon this point were secured by extractions with carbonated water, with a solution of ammonium carbonate and also with hydrochloric acid.

*Solubility of residual magnesium in carbonated water*

In determining the solubility of calcium and magnesium in the soil of the magnesium-treatment cylinders, the sampling of August 15, 1913 was run in parallel with that of May, 1918. The calcium carbonate treatment cylinders

and the control, both sampled in 1918, were also extracted in parallel. Composite samples were made by taking equal amounts from each of 8 magnesium carbonate treatments of both sampling dates, while the same number of subsamples was used in a composite sample of the calcium carbonate treatments. One control composite was made by the use of four no-treatment cylinders and another by the use of eight. A 25-gm. charge of each composite was placed in a 500-cc. cylinder and subjected to agitation by a rapid current of purified  $\text{CO}_2$ , and by frequent shaking. The solutions thus secured after four hours contact were immediately filtered through a 10-cm. Büchner filter, and titrated with 0.05 *N* acid, using methyl orange as an indicator. The titration values and also the determinations of calcium and magnesium thus obtained are given in table 10. It will be noted that the recovery of calcium, as the bicarbonate from the calcium carbonate treatment of the 1918 sampling, was very much greater than that of magnesium from the magnesium carbonate treatment as sampled in 1913, 8 weeks after the incorporation of the treatment. This is the reverse of what would have obtained had both calcium and magnesium carbonates been respectively present in excess. The corresponding disparity between the calcium dissolved from the calcium carbonate treatment of the 1918 sampling and that of magnesium from the 1918 sampling of the magnesium carbonate treatment cylinders was still greater. The calcium carbonate treatment residual was present, however, mainly as the carbonate, while there was no carbonate residual from the magnesium carbonate additions at the time of either the 1913 or the 1918 sampling. The natural leaching to which the cylinders were exposed under humid conditions precluded the accumulation of any appreciable amounts of soluble calcium and magnesium salts prior to the rapid conversion to the air-dry condition. Hence, the solvent action of the carbonated water was exerted in the main upon the residual unabsorbed excess of the precipitated calcium carbonate, in the case of the soils from the cylinders treated with this carbonate; while, in the case of the magnesium treatment, the solvent action was exerted largely as a result of the hydrolysis of the less soluble silicates. There was no great difference between the amounts of calcium dissolved from the untreated soil and that from the two cylinders which received the treatment of magnesium carbonate; however, there was found an appreciable difference between the total titration values and also the occurrences of magnesium. Both the total titration value and the amount of magnesium of the first, or 1913, sampling were greater than the corresponding findings for the second, or 1918, sampling. The amount of magnesium extracted from the precipitated calcium carbonate treated soil was considerably less than that dissolved from the control. This would indicate either one or both of two possibilities—the residual magnesian combinations may have been decreased in the case of the calcium carbonate treatment as the result of an earlier substitution of calcium for magnesium in the siliceous complexes native to the soil; or, the solvent action of the calcium-carbonate-charged carbonated water was less active than the water,

TABLE 11  
*Calcium and magnesium dissolved by simultaneous 4-hour agitation and digestion of 25-gm. charges of composite samples from cylinders treated separately with precipitated  $\text{CaCO}_3$  and precipitated  $\text{MgCO}_3$  by a 10 per cent  $(\text{NH}_4)_2\text{CO}_3$  solution—controls of precipitated  $\text{CaCO}_3$ , precipitated  $\text{MgCO}_3$  and serpentine in parallel. Treatments applied June 13, 1913 at rate of 28,180 pounds  $\text{CaCO}_3$  or equivalent, per 2,000,000 pounds of soil*

MATERIAL APPLIED TO SOIL	DATE OF SAMPLING	CONTROL MATERIAL	CALCIUM			MAGNESIUM		
			As $\text{CaCO}_3$ per 25 gm. of soil	As $\text{CaCO}_3$ in soil	As $\text{CaCO}_3$ per 2,000,000 lbs. of soil	As $\text{CaCO}_3$ per 25 gm. of soil	As $\text{CaCO}_3$ in soil	As $\text{CaCO}_3$ per 2,000,000 lbs. of soil
None* Pptd. $\text{MgCO}_3$ * Pptd. $\text{MgCO}_3$ * Pptd. $\text{CaCO}_3$ *	June 18, 1918		gm.	per cent	lbs.	gm.	per cent	lbs.
	Aug. 15, 1913		0.0003	0.0012	24	0.0046	0.0184	368
	May 6, 1918		0.0003	0.0012	24	0.0895	0.3580	7160
	June 18, 1918		0.0003	0.0116	232	0.0512	0.2048	4096
0.2 gm. $\text{MgCO}_3$ † 0.2 gm. $\text{CaCO}_3$ 0.25 gm. serpentine			0.0003	0.0012	24	0.0131	0.0524	1048
			0.0027	0.0108	216	0.1913	0.7652	15304
			0.0003	0.0012	24	0.0000	0.0000	0
			0.0000	0.0000	0	0.0082	0.0328	656

\* Composite of 8 cylinders.

† Completely dissolved.

relatively free of the calcic salt, upon the magnesium silicates and possibly other complexes. The silicates formed as a result of the absorbed additions may be considered, in one sense, as of the same age in the two samplings. However, the samples taken in 1913 were immediately air dried and remained in that condition during the interval of nearly 5 years, during which time the soil of the 1918 sampling was continually under moist exposure and the dynamic activities of the soil, though it is of course true that the latter sampling had been subjected also to a decreased magnesium content through the process of leaching during the same subsequent period above specified. However, it is indicated quite conclusively that the plants growing in the soil from the magnesium carbonate cylinders were subject most probably to the toxic influence of a larger amount of magnesium, both as bicarbonate and as neutral salts, during the growing season of 1914, than was the case while the plants were growing in the season 1918.

*Solubility of residual magnesium in a solution of ammonium carbonate*

The solubility of the magnesium non-carbonate residuals from the absorbed carbonate treatments was determined by a 4-hour digestion with a 10 per cent aqueous solution of ammonium carbonate, the results of which are given in table 11. The sampling of August 1913 and a corresponding sampling of May 1918 were used, each charge being from a composite of the sampling of 8 cylinders. The precipitated calcium carbonate treatment and the control, both sampled in June, 1918, were used as parallels. Additional controls of 0.20 gm. of precipitated  $MgCO_3$  and the same charge of precipitated  $CaCO_3$  were included, together with a 0.25-gm. charge of finely ground serpentine which had been boiled with dilute hydrochloric acid for one minute in order to remove any trace of carbonate. For convenience of comparison, the calcium and magnesium determinations from these controls were calculated to the soil basis, as though the soil and controls were included in the same charge. It will be noted that the entire 0.20-gm. charge of precipitated magnesium carbonate was dissolved by the ammoniacal carbonate solution. This charge was equivalent to more than double the maximum amount of carbonate dissolved from the soil charges. The amounts of magnesium dissolved by the ammonium carbonate solution were about twice as great as the corresponding extractions by carbonated water, save in the case of the no-treatment cylinder composites. The amount of magnesium dissolved from the composite of the samplings of 1913 was about twice as great as that from the corresponding composite of the 1918 samplings, being in this connection in parallel with the extractions with carbonated water. The ammoniacal carbonate solution was, however, practically inert toward the occurrence of an excess of residual calcium carbonate and upon the control charge of the same alkali-earth carbonate. The fact that more magnesium was extracted from the 1918 composite of the calcium carbonate treatment than from the 1918 sampling of the control may be construed as indicating that the applied calcium carbonate had served to convert

the native magnesium complexes into forms more readily hydrolyzed. The action of ammonium carbonate on the 1913 sampling demonstrates that the non-carbonate residual from the absorbed applied carbonate was still responsive to that solvent. However, the moist condition was maintained for only the initial 8 weeks after the treatment. In the case of the 1918 sampling it is probable that both ageing and leaching over the longer period are controlling factors responsible for the smaller magnesium recovery when simultaneous extractions of the two samplings were made. This relationship is in harmony with that found in the carbonated-water extractions.

TABLE 12

*Solubility of residual calcium and of non-carbonate magnesium in composite samples from cylinders treated separately with precipitated  $\text{CaCO}_3$  and precipitated  $\text{MgCO}_3$ , June 13, 1913,—4 hour boiling digestion with 1-1 HCl, 5-gm. charge*

TREATMENT		DATE OF SAMPLING	CALCIUM		MAGNESIUM	
Material applied to soil	$\text{CaCO}_3 \approx$ per 2,000,000 lbs. of soil		As $\text{CaCO}_3$ in soil	As $\text{CaCO}_3$ per 2,000,000 lbs. of soil	As $\text{CaCO}_3 \approx$ in soil	As $\text{CaCO}_3 \approx$ in 2,000,000 lbs. of soil
	lbs.		per cent	lbs.	per cent	lbs.
None	None	June 18, 1918	0.2510	5020	0.7548	15096
Pptd. $\text{MgCO}_3^*$	28180	Aug. 15, 1913	0.3137	6274	1.0800	21600
Pptd. $\text{MgCO}_3^*$	28180	May 6, 1918	0.2967	5934	1.0120	20240
Pptd. $\text{CaCO}_3^*$	28180	June 18, 1918	1.6487	32974	0.7674	15348

\* Composite from 8 cylinders.

*Solubility of residual magnesium in 1-1 hydrochloric acid*

The amounts of magnesium dissolved from the magnesium carbonate treated cylinders by boiling with 1-1 hydrochloric acid, as compared to those dissolved from the calcium carbonate and untreated cylinders, are given in table 12. Five-gram charges of each composite were boiled for 4 hours with 250 cc. of 1-1 hydrochloric acid in 500-cc. Kjeldahl flasks, fitted with funnels to serve as reflux condensers. The solutions were filtered hot, the residues again suspended in dilute hydrochloric acid and washed upon a 10-cc. Büchner with hot distilled water. The filtrates were evaporated and silica dehydrated. The residue was taken up with dilute hydrochloric acid and the iron thrown out in mechanical combination with the dehydrated silica. The hydrated iron oxide residue was twice re-dissolved, re-filtered and re-precipitated. The combined filtrates from the calcium were evaporated with 25 cc. of nitric acid; taken up; made ammoniacal, manganese precipitated by the addition of bromine and filtered, after which magnesium was determined as magnesium pyrophosphate. The amounts of magnesium thus determined in the charges from the untreated cylinders and those from the cylinders which received the precipitated carbonate of calcium were practically the same. The tendency of the heavy additions of calcium carbonate to liberate magnesium from the soil was offset

apparently by the magnesium content of the heavy application of manure which was made in the case of 4 of the 8 cylinders sampled to secure the composite. The 1913 sampling composite from the 8 cylinders, to which the heavy addition of magnesium carbonate had been made, yielded but 1360 lbs. more per 2,000,000 lbs. of soil than was obtained from the corresponding composite sampled almost 5 years later. This difference is interesting as compared to the much larger corresponding differences of 2240 lbs. and 3064 lbs. obtained by the carbonated water and ammonium carbonate extractions, respectively. The comparison indicates that the differential solubility at the two periods of sampling, as indicated by the two less active solvents, is a more potent factor than is the total, or near-total, amount indicated by the solvent action of the more active acid solvent. The three solvents, carbonated water, ammonium carbonate and hydrochloric acid afford recoveries in magnitude corresponding to the given order. Considering the results secured by these three solvents together with the leaching data, it is evident that, with the advent of fertility, the combined magnesium is now held more tenaciously by the cylinder soil which was subjected to exposure and dynamic conditions for the 5 year period than it was by the same soil which remained under such conditions for only a few weeks.

#### PREVIOUS OBSERVATIONS RELATIVE TO MAGNESIUM-INDUCED TOXICITY AND ITS SUBSEQUENT DISAPPEARANCE

The disappearance of initial toxicity and the advent of fertility, where no assignment of cause can be made to the development of crop tolerance has received little, if any consideration. The problem of the cumulative toxic effect of moderate magnesium additions is distinct from the present consideration of that encountered as the initial effect of single heavy applications. The problem of MgO additions involves factors not involved in  $MgCO_3$  additions; while still another problem arises when we apply either dolomite or magnesite.

The reversion of an excess of the oxide to the carbonate in the soil, in time, makes the usage of the oxide comparable to that of the carbonate. Magnesium oxide additions were made in some of the experiments the results of which are reported in part in this contribution. No seedlings were made, however, where the oxide was added. However, data were secured as to the respective chemical activities of the two forms after their incorporation with the soil. The problem of toxicity caused by accumulations of magnesium carbonate from heavy additions of magnesium oxide and that caused by direct applications of the carbonate will be considered as related and comparable.

It appears that "burned" magnesian lime has been used for a long period in the British Isles. In discussing observations made by Tenant in 1800, and his own experiments conducted in 1806 and 1810, Sir Humphrey Davy (2) states that "a particular species of limestone found in different parts of the North of England, when applied in its burnt and slaked state to land, in considerable quantities, occasioned sterility, or considerably injured the crops for many years," the toxic quality having been assigned to the occurrence of "mag-



nesian earth." Davy concluded that magnesium oxide was slow to carbonate and that toxicity persisted during the continued presence of the uncarbonated oxide; but, that "magnesia in its mild state, i.e., fully combined with carbonic acid, seems to be always an useful constituent of soils." He made mixtures of soil and peat and observed a large formation of carbonate with the decreased proportion of silicates—synonymous with lessened silicate fixation and decreased magnesium solubility. He advocated the use of peat in practice, in order to effect greater carbonation and eradication of toxicity.

In his critical review upon the validity of the experiments reported upon the influence of magnesium as related to the hypothesis of Loew, Lipman (11) aptly stressed "the lack of extensive field experiments," and also, as more germane to our treatise, "the very short period allowed in the different experiments for testing the hypothesis." Hopkins (8) conducted a 5-year pot study relative to the manurial value of magnesium carbonate applied at 5 rates; and the removal of any harmful excess by the leaching of magnesium sulfate formed from additions of calcium sulfate. He found a distinct tendency toward a reduction in toxicity after the third year, even in the case of the 20-ton unleached  $MgCO_3$  treatment without calcium sulfate. This tendency toward improved plant growth in the presence of magnesium accumulations was attributed by Hopkins to the development of a magnesium-resistant strain of wheat. Lipman (11) observed a relation between time and a tendency toward reduced toxicity. He found that an initial toxicity toward citrus trees was mitigated after one and one-half to two years of fallow. Wheeler (34) and Wheeler and Hartwell (35) carried out a field cylinder experiment wherein magnesium oxide was applied, with later supplement of magnesium carbonate. Accounting for an initial toxicity which was followed by a beneficial effect, Wheeler (34) advances the explanation—"The injurious action of the caustic magnesia may, therefore, have been due to the fact that it was too caustic to be endured when in direct contact with the roots of the plants, a condition which was overcome before the succeeding year by virtue of its union with more carbon dioxide," and, "the preceding results show that caustic magnesia was toxic at first when it was used in large quantities, even on a soil evidently in slight need of magnesia, but that when sufficient opportunity had been afforded for it to become carbonated, it became useful." In harmony with our findings relative to the accumulation of neutral magnesium salts from carbonate additions and absence of lethal toxicity, Wheeler and Hartwell found no detrimental effects when neutral magnesium chloride was applied to the soil of the cylinders. Loew (14) contends, however, that the persistence of the magnesium oxide—when magnesium is applied in that form—because of the less rapid reversion to the carbonate, is not the true reason for the toxicity attributed to certain New Jersey limestones.

#### THE TOXICITY INDUCED BY $MgO$ AND $MgCO_3$

The foregoing citations and quotations are in advocacy of the viewpoints; that  $MgO$  was more toxic than an equivalence of  $MgCO_3$ ; that an application of  $MgO$  might be harmful as such, yet beneficial after its conversion to  $MgCO_3$ ; that such carbonation was not rapid, and that the oxide-derived  $MgCO_3$  was considered to be of comparable soil-reactivity with calcium carbonate. A more extensive parallel study of the reversions of  $CaO$  to  $CaCO_3$  and  $MgO$  to  $MgCO_3$  will be considered in another paper. However, the action of an 8-ton  $CaO$ -equivalence of each of the two oxides and carbonates, in both cylinders and lysimeters will be considered here, insofar as the parallel may be directly germane.

Frear (4) treated several soils with  $CaO$  in parallel with  $MgO$  and determined the increase in carbonate accumulations after a period of 10 months. The increase in carbonate was found to have been greater in the case of the  $CaO$

additions than where MgO additions had been made, which led Frear to conclude that the MgO was still, to a large extent, uncarbonated. The intensity of the magnesia-silica reaction had not been pointed out at that time and it is probable that the low carbonate results from the MgO treatments were due in part, at least, to the ready reaction between soil and that part of the MgO which had been converted to magnesium carbonate. Frear wrote later in his classical compilation (5): "In view of MacIntire's results, it is doubtful whether this basic carbonate could accumulate in quantities sufficient to be influential." Schollenberger (30) has offered, more recently, a contribution to this phase of the subject.

Our cylinder results have demonstrated the presence of toxicity from additions of magnesium carbonate, rather than magnesium oxide; and the further fact that the initial toxicity was manifested after the decomposition of the carbonate and the fixation of the magnesium by the soil. The lysimeter studies with the same soil and the same amounts of  $\text{MgCO}_3$ , and also equivalences of MgO, CaO and  $\text{CaCO}_3$ , throw additional light upon the fixation and leaching of the carbonates, both applied and engendered, and the speed of the reversion, to the carbonate. The data of table 13 bear upon the latter point.

The calcic and magnesian additions were mixed throughout the entire amount of soil in the respective tanks. Carbonate- $\text{CO}_2$  determinations were made seven times during the first year and at the end of the second and third annual periods. An unsupported comparison between the determination of residual carbonates derived from chemically equivalent treatments of CaO and MgO at the 8-ton CaO rate would indicate that the CaO had reverted to the carbonate form much more rapidly than had the MgO. The highest  $\text{CaCO}_3$  occurrences from the calcium oxide amounted to .7783 per cent; while the highest  $\text{CaCO}_3$  equivalence from MgO, after the first 10 day interval, amounted to but .1050 per cent in the surface-soil-only series. In like manner, corresponding figures of .8034 per cent and .1143 per cent were the maxima obtained after 17 days in the series which included subsoil beneath the treated surface soil. Without the inclusion of the respective control treatments of equivalent carbonates to establish the parallel as to their respective reactions with the soil, these data would undoubtedly lead to the same conclusions as those drawn by Wheeler and by Frear. However, when the residual carbonates from the precipitated carbonate treatments are included in the comparison, there arises an entirely different deduction, relative to the speed of the respective carbonations of CaO and MgO. An initial carbonate residual of approximately 0.9 per cent for both series is found as a result of the  $\text{CaCO}_3$  additions, as against around 0.3 per cent as an average of the two  $\text{MgCO}_3$  additions, after moist soil and the carbonates had been in contact for 10 days without leaching. The two calcic carbonate treatments decreased slowly in their residual carbonate- $\text{CO}_2$ , until an occurrence of 0.68 per cent  $\text{CaCO}_3$  was found, in both cases, at the end of three years. On the other hand, the magnesium carbonate additions

TABLE 13  
Residual carbonates in terms of  $\text{CaCO}_3$  per acre of 2,000,000 lbs. of soil from analyses of lysimeter soils treated separately with  $\text{CaO}$ ,  $\text{MgO}$ ,  $\text{CaCO}_3$  and  $\text{MgCO}_3$  at the equivalent rate of 8 tons of  $\text{CaO}$  per acre 2,000,000 lbs. of soil. Analyses were made on moist soil but are reported on moisture-free basis.

DATE OF SAMPLING	TIME BETWEEN SAMPLING	SURFACE SOIL ONLY										SURFACE SOIL OVER SUBSOIL									
		CaO					MgO					CaCO <sub>3</sub>					MgCO <sub>3</sub>				
		per cent	lbs.	per cent	lbs.	per cent	per cent	lbs.	per cent	lbs.	per cent	per cent	lbs.	per cent	lbs.	per cent	per cent	lbs.	per cent	lbs.	per cent
7-27-14	10	.....	.....	0.1657	3314	0.8935	17970	0.2939	5878	0.5493	10986	0.8852	17704	0.3609	7218	0.8852	17704	0.3609	7218	0.8852	17704
8-3-14	17	0.4888	9776	0.0818	1636	0.8955	17910	0.2716	5432	0.5811	11622	0.8928	17858	0.3530	7060	0.8928	17858	0.3530	7060	0.8928	17858
8-18-14	32	0.5763	11526	0.1050	2100	0.9115	18230	0.1838	3672	0.6116	12232	0.8966	17932	0.2184	4368	0.8966	17932	0.2184	4368	0.8966	17932
9-4-14	49	0.6657	13314	0.0795	1590	0.8350	16700	0.1483	2966	0.6584	13168	0.8779	17558	0.1975	3950	0.8779	17558	0.1975	3950	0.8779	17558
9-29-14	74	0.7252	14504	0.0625	1250	0.9343	18686	0.1675	3350	0.8034	16068	0.8779	17558	0.1975	3950	0.8779	17558	0.1975	3950	0.8779	17558
2-20-15	218	0.7532	15064	0.0761	1522	0.8877	17754	0.1293	2586	0.8007	16014	0.8704	17408	0.1002	2004	0.8704	17408	0.1002	2004	0.8704	17408
6-18-15	360	0.7783	15568	0.1004	2008	0.8388	16776	0.0994	1988	0.7588	15176	0.8341	16682	0.1090	2180	0.8341	16682	0.1090	2180	0.8341	16682
7-5-16	720	0.6974	13948	0.0253	505	0.7508	15016	0.0228	456	0.6804	13608	0.8341	16682	0.1090	2180	0.7471	14942	0.0332	664	0.7471	14942
7-15-17	1080	0.6410	12820	0.0055*	110*	0.6880	13760	0.0060*	120*	0.6040	12080	0.6830	13660	0.0080*	160*	0.6830	13660	0.0080*	160*	0.6830	13660

\* Probable error in the analysis of a 20-gm. charge amounted to 0.0068 per cent, or 136 pounds.

gave insignificant carbonate occurrences at the end of the same period, the small traces found being within analytical error. The differences in gains of carbonate- $\text{CO}_2$  could not be accounted for by the greater solubility of the engendered  $\text{MgCO}_3$ , as compared to that of the  $\text{CaCO}_3$  formed, and the greater consequential leaching of  $\text{MgCO}_3$ ; for, the total magnesium leached, both as neutral salts and as bicarbonates, amounted to but 5486 pounds from the  $\text{MgO}$  added to the surface soil. Of this amount, only 3312 pounds came through as bicarbonates; while, of the total leaching of all forms, amounting to 7298 pounds, in the parallel application of  $\text{MgCO}_3$ , only 5296 pounds were to be accounted for by the presence of bicarbonates. The average of the nine determinations to  $\text{CaCO}_3$ -equivalent residual from the precipitated calcium carbonate was 16978 pounds during the first three years, in the case of the surface-soil-only series, as against 2938 pounds for the magnesium carbonate. On the other hand, corresponding values of 16993 pounds and 3613 pounds, respectively, were obtained from the soil treatments in the subsoil series. It is apparent, therefore, that the large carbonate residual derived from the added  $\text{CaO}$  accumulated not alone because of more rapid carbonation, but because of the additional fact that the calcium carbonate was not so quickly, nor so extensively, fixed by the soil. The analyses of the leachings from the lysimeters showed that the magnesium treatments caused a somewhat larger outgo of nitrates and sulfates and this factor and that of the greater solubility of the magnesium carbonate account for part of the disparity between the residuals of the carbonates of magnesium and calcium.

It is true that  $\text{MgO}$  hydrates less readily than does  $\text{CaO}$  under exposure to normal atmospheric conditions, and the subsequent conversion to the carbonate is, therefore, less speedy because of this less rapid conversion to the essential preliminary combination under the stated conditions. However, in suspension of the two oxides in carbonated water at ordinary laboratory temperature, where the supply of  $\text{CO}_2$  is maintained by steady influx at atmospheric pressure, the amount of magnesium carbonate formed and ultimately dissolved as bicarbonate, is approximately thirty times as great as that of calcium bicarbonate. Such a condition would be approached more nearly by the soil moisture than through ordinary exposure to the atmosphere. Thus, particularly in the case of heavy additions, we would expect to find that while the magnesium oxide applied to the soil might be reverted to the carbonate more slowly than would lime, as a result of less rapid hydration essentially precedent to carbonation, the soil solution would soon become more heavily impregnated with magnesium bicarbonate than with calcium bicarbonate. The leaching data proved the correctness of this assumption.

Because of the intimate mixture of soil with oxide, hydroxide, interspersed granules of carbonate and freshly formed silicates in the lysimeters, it would be difficult to assign definite approximate values to the several magnesian combinations as sources of the leached magnesium. Variations in the amounts of  $\text{CO}_2$  liberated from the carbonate form, and thus made available for either

the further carbonation of the uncarbonated hydroxide, or for the more extensive hydrolysis of the magnesian siliceous complexes would, of course, materially affect both the total amount and the form of the leached magnesium. Considering the ready solubility of the pulverulent carbonate, and the strong tendency of this compound to be fixed by the soil, together with the ready conversion of the hydroxide—always a constituent of the basic carbonate—it would seem logical to conclude somewhat as follows. The  $\text{MgCO}_3$  would react with the acid silicates and with biologically engendered acids, causing a liberation of  $\text{CO}_2$ . This would be taken up in large part by the  $\text{Mg}(\text{OH})_2$ , with the further fixation of  $\text{MgCO}_3$ , or leaching of the resultant bicarbonate. After the conversion of the hydroxide to the carbonate, there would still be dynamic activities wherein  $\text{CO}_2$ , liberated by decomposition of absorbed magnesium carbonate would then tend to form carbonated water, which would be effective in causing the reversal of the fixation process through hydrolysis and carbonation of the hydrolyzed derivative from the silicate. But, as indicated by the leaching data, the magnitude of the products of such dynamic conditions and the tendency toward electrolytic dissociation both approach the minimum with increase of time.

#### THE BEARING OF TRANSIENT TOXICITY UPON LIME-MAGNESIA RATIO STUDIES

The pronouncement by Loew (12) of his optimum lime-magnesia ratio hypothesis and his subsequent ardent advocacy of his viewpoint inspired a number of investigations upon the subject. The biological phase of the problem and the possible antagonistic relationships between calcium and magnesium have been also extensively studied. A number of studies have been carried out in water cultures, to the exclusion of the vitiating influence of the solid phase, soil mass or quartz sand. The late Dr. Wm. Frear (5) very aptly drew attention to the fallacy of applying results obtained in water cultures to the practice of liming with calcareous and dolomitic products, when he wrote—"From the fact of water culture experiments with calcium and magnesium chlorides, nitrates and sulfates, to those of the influence of calcareous or magnesium dressings, as oxides, hydrates or carbonates upon cropped land, is a very long road with many streams to cross." Through citations, McCool (25), also, has stressed the function of the solid media in ameliorating the tendencies exerted by the soluble alkali and alkali-earth salts. Such water culture experiments are not considered by us as being closely related to the soil studies offered in this contribution.

The findings reported in this paper were not obtained in an effort to determine the persistency of magnesia-induced toxicity, nor in a study of the Loew hypothesis, as such. It is not intended, therefore, to offer an extensive survey of the lime-magnesia investigations. Such has been done in the bibliographies compiled by Loew (13, 14), Lipman (11), Gile (6) and McCool (25). However, the results relative to the differential extent of  $\text{CaCO}_3$  and  $\text{MgCO}_3$  fixations, and the differences between  $\text{CaO}$  and  $\text{MgO}$  transformations into carbonates in the atmosphere as compared with such transformations in the body of the soil were not known when many of the earlier experiments were carried out. Furthermore, the studies were usually of such brief duration that it had not been observed that an initial toxicity might be converted later into a beneficial effect, with but little difference in the amounts of magnesium present under the two conditions. These later findings introduce factors which were not taken into account in many of the earlier related investigations.

In those instances where the equivalent alkali-earth carbonate additions were in such small amounts that all of both calcium and magnesium carbonates were fixed, the variations between

the solubilities of the fixed derivatives may not have been of great import. But, when the additions were heavy and of chemical equivalence, the unabsorbed  $\text{CaCO}_3$  residuals may have been compared with magnesium silicate complexes, rather than a residual of magnesium carbonate. In such cases, the original comparative solubilities of the two carbonates do not apply, but rather a new and reverse order of solubility. Again, it has been shown (19, 23) that  $\text{MgO}$  will quickly revert to the carbonate in suspensions of carbonated water. However, with less opportunity for diffusion of the weaker carbonated solution, the magnesium carbonate will be formed not so readily in the soil and it is there subjected to both leaching and fixation. However, this chemical parallel is not complete in the case of sand cultures. Hence the plant growth results from  $\text{CaO-MgO}$  comparisons in sand culture experiments may not be properly considered as having unqualified application to soils, even with the exclusion of biological influences. Again, we would stress that unfortunately many of the previous experiments were of but brief duration, affording no opportunity to establish the fact that the initial effects may not be necessarily permanent.

Loew (13, 14) pointed out that the variation in the solubilities of the several solid-phase carbonates, as shown by Ulbricht, was to be considered as an *a priori* factor in ratio studies, drawing particular attention to the difference between the pulverulent precipitated carbonate and pulverized magnesite. He also contended that the toxic influence of the basic precipitated carbonate is due to the presence of the hydrate which, however, he maintained would soon be converted to the carbonate. Daikuhara (1) took cognizance of the disparity between the intensity of the influences exerted by the soluble sulfate of magnesium and that exerted by the precipitated carbonate of the same element in antagonistic action upon precipitated calcium carbonate present in sand cultures, wherein rice was grown. Nakamura, (28) in studying magnesium deficiency, makes an interesting observation to the effect that, "the proper way to correct the unfavorable ratio of lime and magnesia of this soil, for the growth of Gramineae, would be to add so much magnesia in the form of insoluble hydrous silicate, or powdered magnesite that the ratio of lime to magnesia becomes 1:1, for the availability of the magnesia would then agree or nearly so with that of the lime."

Our viewpoint, suggested by the data given, is that the silication of the large amounts of magnesium carbonate, which some soils can fix, represents an approach toward the native aged mineral silicates. Nakamura further differentiated between the soluble sulfate and powdered magnesite giving an "agronomical ratio" of 23:100 and he pointed out that an addition of the soluble sulfate should be very much less for a sandy than for a clay soil. Loew and Aso (15) likewise maintained that the proper conception of optimum lime-magnesia ratio is based upon the "condition that the availability is equal," the context indicating that the primary consideration entertained by them was the solubility of the applied material, *per se*. However, in discussing the importance of availability coefficient, which they designate as "agronomical equivalent," they do state that "this magnitude changes with the nature of the soils and the partial transformation of the applied compounds into other forms in the soil". Meyer (26) concluded that the deleterious effect brought about by repetitions of magnesia additions were the result of the strong alkaline reaction induced by the added oxide. The hydrated oxide of magnesia is, however, exceedingly insoluble, as compared with hydrated lime and only a small amount of the strongly dissociated hydroxide would be present in the free soil-water, so long as the caustic form persists. But, after reversion of  $\text{MgO}$  to  $\text{MgCO}_3$ , the magnesium is in a form about 30 times as soluble as that of the corresponding physical form of calcium. Meyer (26) found that magnesia of burned dolomitic limestone and marl to be less harmful than the magnesium oxide derived from calcined magnesite. He concluded that the practical use of dolomitic lime is dependent upon soil's texture and its store of acid-reacting materials, the heavier and more acid a soil, the greater the permissible application of the  $\text{CaO-MgO}$ , while its use on a sandy soil is deemed not advisable.

As previously stated, it was pointed out by Loew that the pulverulent precipitated magnesium carbonate, which invariably carried some magnesium hydrate, is many times more soluble and consequently more toxic than the native magnesite when applied to soils; but, it

has not been generally accepted that the same variation between the solubilities of precipitated calcium carbonate and finely ground limestone is of sufficient magnitude to be accorded serious consideration. It has been shown, however, by Mooers and MacIntire (27) that the precipitated calcium carbonate is more active than a chemical equivalence of a practical limestone mixture in causing a loss of soluble nitrogen from the soil used in the cylinder experiments reported in this paper.

In a large number of pot studies the calcium and magnesium additions have been in the form of precipitated carbonates, the oxides being used less frequently. It seems to have been tacitly assumed, as a rule, that the chemical activities of the directly applied, or oxide-derived, carbonates of the two elements were of equivalent, or approximate, chemical activity, with a maintenance of their respective solubilities after incorporation with the soil. Again, in the case of heavy applications of precipitated  $\text{CaCO}_3$  and  $\text{MgCO}_3$  in chemical equivalence, it was tacitly assumed that both the initial disintegration and the residual occurrences of the two carbonates were in exact, or near, chemical equivalence. This assumption was also proved to be erroneous by the writer and associates (19; 23) who found a marked difference between the speed of the lime-silica reaction and that involving the union of magnesia with silica, as well as an inverse relationship in the speed of the reversal of the reactions through hydrolysis. Little, if any control has been applied to either the differential extent or speed of the reactions responsible for the carbonation, absorption, or fixation phenomena and the variation in the characteristics of the precipitated end-products. In a word, most of the pot and cylinder investigations were carried out without periodic determinations of the disparity between carbonate residuals from the applications of the oxides and carbonates of calcium and magnesium. Neither was much attention paid to the differential tendencies of the absorption end-products to undergo reversal of form, when in contact with carbonated water carrying soil nutrients. The problem has been viewed generally from the angle of the relative effect produced by the two elements through the medium of the applied basic materials, *per se*, or their salts representing biological end-products, rather than those effects induced by their ultimate and more stable combinations in the soil. Little attention was paid to either the distinct variation in the speed of the reactions incident to fixation, or the decided differences in the respective complexities of the engendered silicates of the two alkali-earth bases. Nevertheless, these factors are responsible for chemically characteristic variations in their respective tendencies toward hydrolysis, the initial step in the reversal of the reaction by which, in the absence of the carbonates in the solid phase, the soil solution becomes impregnated with calcium and magnesium bicarbonates. Frear (5), however, apparently had in mind a concept in harmony with the viewpoint which we believe our findings justify, relative to ratio studies, when he wrote: "Either calcium or magnesium brought to the soil in a given state of combination will probably undergo a change of combination after it has been exposed to the soil, and some of it may remain in solution, while another portion is converted to an insoluble compound."

Some of the findings reported in this contribution have a very definite bearing upon the validity of the hypothesis of an approximate optimum lime-magnesia ratio. Loew (13) contends that, "it is always an unfavorable condition when the magnesium content of a soil is essentially higher than the lime content." The results of tables 8, 10, 11 and 12 do not sustain such a contention. Table 8 shows that a large proportion of the added magnesium carbonate is still present, though not in carbonate form, after 5 years. Table 12 shows the residual magnesium to be extensively soluble in hydrochloric acid. Table 11 demonstrates the ready and preponderating solubility of the non-carbonate magnesium residuals in solutions of ammonium carbonate.

But, special stress may be placed upon the data of table 10, since the solubility of the remaining calcium carbonate and that of the absorbed magnesium

residuals in carbonated water is the nearest approach we have to the solvent action of the free soil water which brings supplies of calcium and magnesium to the plant roots. The soil which received precipitated calcium carbonate had become much more productive than the untreated soil at the time of the 1918 sampling. But, with increased productivity, there had come a marked change in the carbonated-water soluble lime-magnesia ratio, an increase from that of 1 to 1.6 to that of 28.7 to 1. Conversely, the magnesium additions had produced definite fertility in 1918, yet they had altered the carbonated-water soluble lime-magnesia ratio from that of 1 to 1.6 to that of 1 to 4.5. Thus, the soil of an original carbonated-water-soluble lime-magnesia ratio of 1 to 1.6 was greatly benefited both by the change to a ratio of 28.7 to 1, and by that of an alteration from 1 to 1.6 to 1 to 4.5. The magnesium additions materially altered the original ratio and produced an excess of both total and available magnesium, the condition termed "unfavorable" by Loew, yet improved growth resulted from the alteration.

In studying lime-magnesia relationships by means of pot or basket experiments, it should be realized that sand (silica) and soil (silicate complexes) are distinctly different media. The two materials vary widely in their abilities to offset the effect of the variation in the active mass resultant from the differential solubilities of the precipitated carbonates of calcium and magnesium in both pure and carbonated water. The silicate complexes are much more active than silica in bringing about an extensive precipitation and fixation or "locking up" of magnesium.

It is readily seen that a moderate or heavy treatment of precipitated  $\text{CaCO}_3$  or  $\text{MgCO}_3$  in sand culture would remain largely in the carbonate form, a form somewhat soluble; while in the case of a soil medium the incorporated bases would enter, with distinctly varying degree, into silicate combinations of varying complexity—forms not so readily soluble in the case of absorbed magnesia. It is, of course, recognized that the simple calcium silicate,  $\text{Ca}_2\text{Si}_2\text{O}_6$ , is readily hydrolyzed, assuming the improbable formation of so simple a compound, rather than the more probable complex silicates, when lime is fixed by the soil. The texture of the soil is, of course, a factor in the speed of the silication process. The finer separates run parallel quantitatively with greater complexity of silicates, and this tends toward more intensive, speedy, and involved fixation of alkali-earth bases than would be the case with the relatively pure silica of the quartz sand medium. Hence, we find insufficient stress laid on the inherent differences between pot or basket work with sand, a comparatively inert medium *at ordinary temperatures*, and similar work with soils carrying varying amounts of the more active acid clay, in large measure finely divided silicates. Without doubt, it is hardly permissible to use the water culture results relative to calcium and magnesium salt relationships, as having unqualified application to plant growth in soils, and we believe the same to be true also in the case of results secured from sand cultures. Our conclusion is that cultures in the relatively inactive acid-silicate-free sand



should not be considered as comparable with lime-magnesia ratio studies in soil media where the different silicates show so much more marked tendency toward the rapid decomposition of the applied carbonates, the rapidity and extent of the fixing of magnesium carbonate being much greater than that of the carbonate of calcium. It seems logical to assume that somewhat different conclusions and practical applications might have come from some of the earlier investigations had these facts been given consideration in the control and interpretation of those experiments.

#### SUMMARY

1. Cylinder studies upon tall-oat grass and cowpeas over a period of years are reported. There prevailed during the initial year a toxicity lethal to three seedlings of tall oat grass where heavy additions of precipitated magnesium carbonate had been made, though the carbonate additions had been completely decomposed. This initial toxic condition was succeeded by a beneficial effect.

2. No toxic effect was produced upon either tall oat grass or cowpeas by heavy additions of dolomite, which proved to be about as valuable as limestone of similar fineness and chemical equivalence.

3. Repetition of the cylinder work, as to initial toxicity induced by magnesium carbonate additions upon cowpeas, was made by the use of Wagner pots. Initial toxicity to cowpeas was found; but, this had disappeared at the end of the first year in the unleached pots. A large preponderance of magnesium salts over those of calcium was found in the several zones when the soils were leached at the time of harvesting of the cowpeas, at which time there was no indication of toxicity.

4. Samplings from the cylinders were made in 1913 and 1918 and the pH values of distilled and carbonated water extracts were determined in 1922. The aged magnesian non-carbonate residuals gave values indicating acidity, while the reverse was true in the case of the calcium carbonate residues.

5. Possible reasons for the initial toxicity and its subsequent disappearance were discussed. The data suggested that most probably the aging of the absorbed magnesia and the probable formation of more complex precipitates were responsible for less ready solution in the soil water and for decreased hydroxyl-ion concentrations.

6. Leaching data were given showing the annual and total outgo of magnesium and the progressive decrease in solubility of magnesian additions during the five-year period, in the case of soil used in the cylinders. These additional data established the fact of an occurrence of a large residue of non-carbonate magnesium in the cylinders during the period of lethal toxicity and subsequent fertility. They also determined the forms in which the magnesium was leached from the soil during the prevalence of these two conditions.

7. Information was also obtained relative to the reversible liberative action of excesses of both calcium and magnesium in subsoil, and the effect of the subsoil in stopping the outgo of the two alkali-earth bases.

8. The relative solubility of the cylinder magnesium residuals in the samplings after the first and fifth years was determined by carbonated water, aqueous ammonium carbonate and hydrochloric acid extractions. The large recovery of the unabsorbed  $\text{CaCO}_3$  and the small recovery of magnesium from the non-carbonate forms in the carbonated water extracts and the variations between samplings are discussed as indicating the influence of the aging of the magnesium complexes. The reverse order of solubility of calcium and magnesium in aqueous ammonium carbonate and the solvent action of  $\text{HCl}$  are also discussed.

9. The earlier observations relative to toxicity induced by magnesium oxide and precipitated basic magnesium carbonate were considered in the light of the findings relative to the extensive fixation of magnesium by the soil.

10. The parallel between the supposed persistence of applied magnesium oxide and the gradual accumulation of magnesium carbonate, as being responsible for the observed cases of initial toxicity and its subsequent disappearance, were considered in the light of initial toxicity observed by us after the absorption of the applied magnesium carbonate and the later change to a beneficial effect.

11. Residual  $\text{CO}_2$  data and leaching results are given showing that the slow hydration and carbonation of  $\text{MgO}$  in the atmosphere is altogether different from the problem of  $\text{MgO}$  conversion to carbonate in the body of the soil.

12. The results of the experiments given in this contribution are considered in their bearing upon some of the previous investigations upon the validity of the so-called optimum lime-magnesia ratio hypothesis of Loew. It was pointed out that most of the previous experiments were continued for such brief periods that only the initial effects were observed.

13. Attention is called to the fallacy of using sand cultures as the medium for plant growth, in studies upon the activities of the oxides or precipitated carbonates of calcium and magnesium, when findings so obtained are to be considered as applicable to soils. This, because of the relatively small extent and minimum speed of the reaction between silica and magnesia, as compared to that of the greater extent and speed of the reaction between acid silicates and magnesia.

14. Special stress is laid upon the fact that the comparison of the availabilities of calcic and magnesian additions should not be made upon the basis of the solubilities of the original carriers, but rather upon the solubilities of the absorbed end-products and the unchanged residuals, both of which vary according to the base applied, the form in which they are added, and the period intervening between treatment and analysis.

15. Data are submitted showing that with wide alternating ranges of solubilities of calcium and magnesium, there appeared to be no correlation in the effects produced upon plant growth.

16. It was pointed out that if plant culture experiments relative to the effects of the more active forms of magnesium are conducted over a period of

years, it may be true in many cases, that an initial period of toxicity may be converted into a period of beneficial results—as was found to be the case in our experiments.

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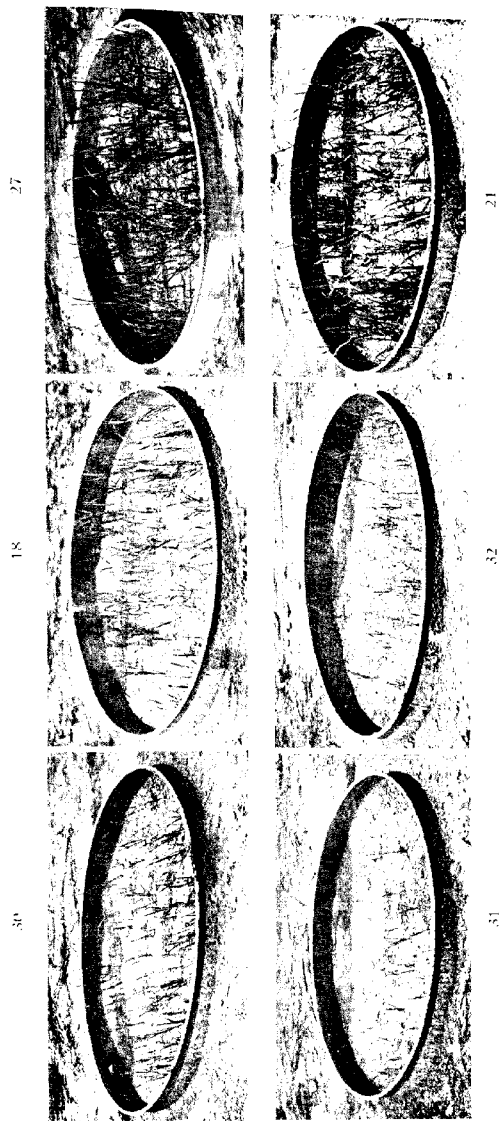
PLATE 1

SHOWING INITIAL TONICITY INDUCED BY NON-CARRONATE RESIDUES FROM ADDED PRECIPITATED MAGNESIUM CARBONATE AT THE RATE OF 28180 POUNDS  $\text{CaCO}_3$  EQUIVALENCES PER 2,000,000 POUNDS OF SOIL

Treatments June 11, 1913. Seeding April 7, 1914. Photographed May 27, 1914, just before disappearance of growth on cylinders 31 and 32. Compare with plates 2 and 3.

*Treatments per 2,000,000 lbs. soil. Series X*

30	18	27
No treatment	30 tons stable manure	28,180 lbs. $\text{CaCO}_3$
31	32	21
$\text{MgCO}_3$ equivalent to 28,180 lbs. $\text{CaCO}_3$	$\text{MgCO}_3$ equivalent to 28,180 lbs. $\text{CaCO}_3$ and 30 tons stable manure	28,180 lbs. $\text{CaCO}_3$ and 48 tons stable manure



## PLATE 2

COMPARISON BETWEEN EFFECT OF RESIDUAL PRECIPITATED CALCIUM CARBONATE AND THE  
NON-CARBONATE RESIDUALS FROM MAGNESIUM CARBONATE UPON TALL OAT GRASS  
DURING PERIOD OF FERTILITY FOLLOWING INITIAL PERIOD OF MAGNESIUM-INDUCED  
TOXICITY

Cylinders seeded April 7, 1914. Growth on cylinders 31 and 32 from fourth seeding,  
Oct. 26, 1914. Photo June 5, 1917.

*Treatments per 2,000,000 lbs. soil. Series X*

30	18	27
No treatment	30 tons stable manure	28,180 lbs. $\text{CaCO}_3$
31	32	21
$\text{MgCO}_3$ equivalent to 28,180 lbs. $\text{CaCO}_3$	$\text{MgCO}_3$ equivalent to 28,180 lbs. $\text{CaCO}_3$ and 30 tons stable manure	28,180 lbs. $\text{CaCO}_3$ and 30 tons stable manure.

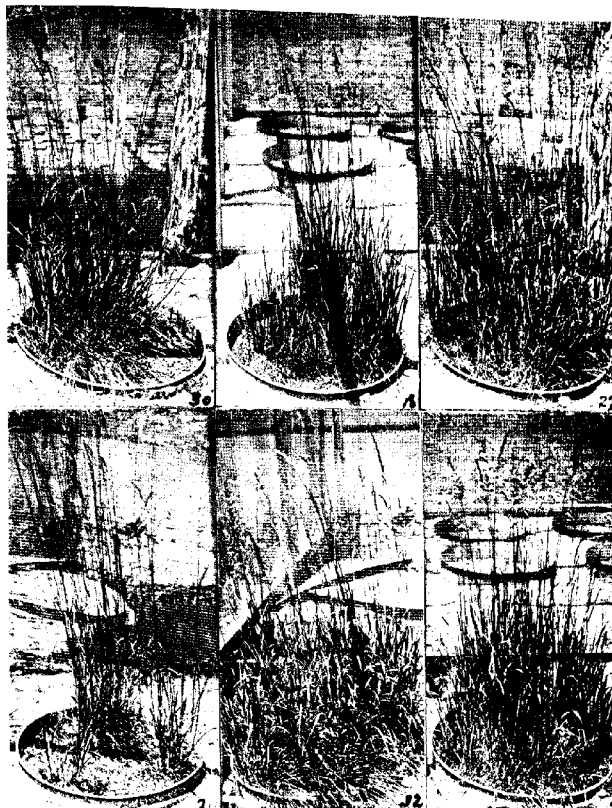




PLATE 3

SHOWING ABSENCE OF INITIAL MAGNESIUM-INDUCED TOXICITY, EARLY STAGE OF 1917 CROP

Treatments June 11, 1913, seeding April 7, 1914. Fourth seeding, responsible for growth in cylinders 31 and 32, made Oct. 26, 1914. Photo made March 19, 1917.

*Treatments for 2,000,000 lbs. soil. Series X*

30	18
No treatment	30 tons stable manure
31	32
MgCO <sub>3</sub> equivalent to 28,180 lbs. CaCO <sub>3</sub>	MgCO <sub>3</sub> equivalent to 28,180 lbs. CaCO <sub>3</sub> and 30 tons stable manure



PLATE 4

SHOWING ABSENCE OF MAGNESIUM-INDUCED TOXICITY UPON COMPEX CROPS 1917

Seeded June 16. Harvested September 4, 1917.

*Treatments per 2,000,000 pounds of soil. Series K.*

30	18
No treatment	30 tons stable manure
31	32
MgCO <sub>3</sub> equivalent to 28,180 pounds CaCO <sub>3</sub>	MgCO <sub>3</sub> equivalent to 28,180 pounds CaCO <sub>3</sub> and 30 tons stable manure



PLATE 5

SHOWING INITIAL TOXICITY INDUCED BY TREATMENTS OF  $\text{MgCO}_3$  EQUIVALENT TO 28180  
POUNDS  $\text{CaCO}_3$  PER 2,000,000 POUNDS OF SOIL IN WAGNER POTS, AND ITS DISAPPEARANCE  
AFTER ONE YEAR

- A. Treatment and seeding Aug. 3, 1918. Photo made in chlorotic stage Sept. 6, 1918.
- B. Treatment Aug. 3, 1918, seeding Sept. 24, 1918. Photo made in chlorotic stage Oct. 23, 1918.
- C. Treatment Aug. 3, 1918, seeding Sept. 3, 1919. Photo made in chlorotic stage, Oct. 2, 1919.

*a*



*B*



*C*





## A NOTE ON SOIL REACTION STUDIES<sup>1</sup>

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For certain lines of investigational work of the Experiment Station, samples of soil have been collected from different plots of a number of the soil fertility experiment fields in the state. The lime requirement of these samples was determined shortly after collection by the Veitch and Hopkins methods. Lately the hydrogen-ion concentration has been determined by both the electrometric and colorimetric methods.

In the summer of 1916 Professor George Roberts made a field study of the growth of sweet clover in a section of the state where this is an important crop. The soils in this section are largely residual from predominatingly limestone formations. Up to the present but little attention has been paid to the reaction of the soils and the use of lime where necessary to insure successful growth of the crop. There have been wide variations in the success of the crop depending on the extent to which the original basic materials have been removed from the surface soil layers and their resulting reaction. In connection with this field study, samples of soil were taken from areas representing varying degrees of success of growth of the crop for further studies in the laboratory. Lime requirement determinations by the Veitch and Hopkins methods, and litmus paper and HCl reaction tests were made shortly after collection. Hydrogen-ion concentrations have also been determined lately by the colorimetric method.

The results from these reaction determinations, heretofore unpublished, are presented in this paper in connection with the yields of red clover in the field in the case of the experiment field plots and with the nature of the sweet clover growth indicated in the case of the sweet clover study samples.

For the experiment field soils only the pH values obtained by the electrometric method are given. The readings were made with a Leeds and Northrup student potentiometer. The hydrogen electrode vessels used were made in the laboratory. They were 3.4 cm. in diameter and 3.5 cm. in height, inside measurements. Clark hydrogen electrodes were used. The stopper carried the usual entrance and exit tubes for hydrogen, and connecting tube for calomel electrode. The hydrogen electrodes were cleaned and coated with platinum black, generally after every third determination. The calomel electrode was also made in the laboratory. Hydrogen from zinc was used for the most

<sup>1</sup> Contribution from the Department of Agronomy, Kentucky Agricultural Experiment Station. Published with permission of the Director.



TABLE 1  
Reaction and clover yields on experiment field soils

PLOT NUMBER	TREATMENT*	REACTION (ELECTROMETRIC)	CaCO <sub>3</sub> REQUIREMENT PER ACRE		YIELD OF CLOVER HAY PER ACRE†
			Veitch method	Hopkins method	
		pH	lbs.	lbs.	
Lexington Field					
403	R*	5.69	2800	0	4582
408	RL	6.23	1800	0	4293
401	RAP	5.78	3400	0	4478
406	RLAP	6.47	600	0	4268
Russellville Field					
201		5.12	2340	240	1399
213	M	4.95	2480	360	1578
202	ML	6.92	0	0	2686
207	MLAP	6.80	0	0	3530
Greenville Field					
201		5.07	1600	24	135
203	M	5.54	1000	76	843
202	ML	6.92	0	0	1555
207	MLAP	6.48	0	0	2831
Mayfield Field					
201		4.68	3200	920	763
203	M	4.84	2600	820	978
202	ML	6.96	0	0	2848
204	MAP	4.59	3200	1120	1052
206	MLAP	6.75	0	0	3858

\* R = residues

M = manure

L = limestone

AP = acid phosphate

The rotation used on these fields has been corn (with cover crop) soybeans, wheat, and clover. There are four series of plots to make possible the growth of all the crops each year. The applications of limestone and acid phosphate have been at the rate of 2 tons and 800 pounds respectively, per acre per rotation applied before the corn crop. On the fields where the manure treatments are used, the corn and wheat grain, the corn stalks, wheat straw, soybean and clover hay are removed. Beginning with 1916 manure has been applied once per rotation before the corn crops at the rate of 6 tons per acre, with the exception of the Berea field where the first application was in 1917. At the time of sampling, the plots had each had one application of manure. On the fields where the residue treatments are used, the corn, wheat and soybean grain and the clover hay are removed. The corn stalks and wheat and soybean straw are returned to the land. Fuller information as to the plan of conducting and results from these fields is given in Kentucky Experiment Station Bulletin 228 and Circular 123 (1, 2).

† Yields are averages of the four series usually from the beginning of the experiments through the 1922 yields, a period of 5-7 years on the various fields.

TABLE 1—Continued

PLOT NUMBER	TREATMENT	REACTION (ELECTROMETRIC)	CaCO <sub>2</sub> REQUIREMENT PER ACRE		YIELD OF CLOVER HAY PER ACRE†
			Veitch method	Hopkins method	
		pH	lbs.	lbs.	lbs.
<i>Berea Field</i>					
101		4.91	1200	780	68
102	ML	6.40	0	0	660
104	MAP	4.77	2400	1220	706
106	MLAP	6.82	0	0	2058
<i>Fariston Field</i>					
302		4.52	1400	1280	19
303	RL	6.29	0	0	323
308	RAP	4.62	1400	1220	842
311	RLAP	5.78	200	0	1599

TABLE 2

*Reaction and clover yields on four experiment field soils*

TREATMENT*	PLOT NUMBER	REACTION (ELECTRO- METRIC)	YIELD OF CLOVER HAY PER ACRE†	PLOT NUMBER	REACTION (ELECTRO- METRIC)	YIELD OF CLOVER HAY PER ACRE†
		pH	lbs.		pH	lbs.
<i>Russellville Field</i>				<i>Mayfield Field</i>		
M	304	4.93	1578	307	4.95	978
ML	302	6.86	2686	302	7.35	2848
MAP	303	4.97	2299	304	4.80	1052
MRP	306	4.93	2791	305	5.13	1818
MLAP	307	6.94	3530	306	6.98	3858
MLRP	308	7.05	3433	308	7.19	3604
<i>Berea Field</i>				<i>Fariston Field‡</i>		
M	203	4.50	374	207	4.45	113
ML	202	6.41	660	203	5.82	323
MAP	204	4.35	706	208	4.63	842
MRP	205	4.57	717	205	4.56	794
MLAP	206	6.20	2058	211	5.85	1599
MLRP	207	5.99	1849	214	5.70	811

\*M = manure

L = limestone

AP = acid phosphate

RP = rock phosphate

Limestone and acid phosphate applications same as indicated in table 1. Rock phosphate applied at rate of 1600 pounds per acre per rotation before the corn crop.

† The clover yields are the same as those in table 1 except for the addition of the yields on the RP and LRP plots.

‡ Crop residues instead of manure have been used on the Fariston field.

part. Certain readings were repeated with tank hydrogen from the Will Corporation prepared for hydrogen-ion work. The readings were in good agreement.

Ten grams of soil and 15 cc. of water were used per reading. The vessels were hand shaken in so far as additional agitation was necessary over that given by the bubbling of hydrogen. The readings on most of these soils were made by Wm. M. Phipps, a student doing some special work in soils, as well as by the writer. Differences in readings due to the personal equation were not important. A difference of pH .05 was the maximum variation in duplicate readings. The duplicate readings were made at the same time using two hydrogen electrode vessels. No difficulty was experienced in getting this agreement when the outfit was in satisfactory working condition. Fifteen to twenty minutes was the time usually required for constant potential to be reached. Corrections were made for variations in temperature.

With the exception of the Lexington samples, the samples reported in table 1 were taken in October, 1920. The Lexington samples were taken in December, 1920. At the time of sampling, the plots were in clover seeded in the preceding spring, in wheat. The Hopkins and Veitch determinations were made at various times from December, 1920, to March, 1921. The hydrogen-ion concentration readings were made for the most part in August, 1922.

Soil samples were also taken from the Mayfield and Russellville fields in July, 1921, and from the Berea and Fariston fields in December, 1921. At this time the Russellville, Mayfield, and Berea fields were in clover, seeded in small grain in the preceding spring. The Fariston field had been in corn in 1921 and was in a cover crop of rye at the time of sampling. The hydrogen-ion concentration of these samples was determined electrometrically by J. F. Freeman, graduate student, in September, 1922, using the same apparatus and procedure as in the preceding determinations. The results are given in table 2. For the most part differences in time of sampling and in series sampled have had but little effect on the pH values as shown in tables 1 and 2. These values were determined after the samples had been in the air-dry condition in the laboratory for some time. It is possible that readings on the fresh samples might have been somewhat different.

Limestone is the important fertilizer treatment in affecting hydrogen-ion concentration and resulted in pH values of 6.5-7.0, except on the Lexington and Fariston fields. It is noteworthy that the Lexington field where the unlimed soil gives the highest pH value is the one where, on the whole, limestone has been of least effect in raising pH value. This may be accounted for by assuming it to be a more highly buffered soil because of its larger organic matter content. Where applied without limestone, rock phosphate has given considerably larger yields of clover as well as of other crops than has acid phosphate, but the effect of the two materials on hydrogen-ion concentration has been very slight if any.

TABLE 3  
Sweet clover study soils arranged in order of hydrogen-ion concentration of surface soils

SAMPLE NUMBER*	REACTION (COLORIMETRIC METHOD)	CaCO <sub>3</sub> REQUIREMENT		REACTION FROM LITMUS PAPER TEST§	EFFERVESCENCE ON ADDING HCl	NATURE OF SWEET CLOVER GROWTH
		Veitch method	Hopkins method			
	pH	lbs.	lbs.			
2	7.63	alk.	100	neut. to alk.	strong	good
2a†	7.63	alk.	160	neut. to alk.	strong	
1	7.00	alk.	alk.	neut.	strong	good
1a	5.65	....	60	sl. § acid	doubtful	
15	7.00	alk.	10	neut.	medium	good
15a	5.15	1365	440	acid	none	
18	6.35	alk.	70	neut.	medium	good
18a	7.40	alk.	40	neut.	medium	
9	6.40	alk.	40	....	....	good
21	6.00	....	40	v. sl. acid	medium	good
21a	....	....	80	sl. acid	strong	
22	5.70	....	40	v. sl. acid	medium	good
22a	7.63	....	50	neut.	strong	
4	5.60	alk.	90	neut.	none	good
4a	4.90	1911	680	neut.	none	
7	....	alk.	120	neut.	none	good
7a	5.30	1465	....	v. sl. acid	none	
10	5.40	1638	40	neut.	medium	good
10a	5.20	4641	1580	acid	medium	
6	5.45	637	70	neut. to alk.	none	poor
6a	4.90	5369	4580	str. acid	slight	
11	5.05	910	140	sl. acid	none	almost none
11a	5.45	2366	1080	acid	none	
12	4.95	1911	500	acid	none	none
12a	5.15	....	1360	str. acid	none	
8‡	....	2912	1300	acid	none	poor
8a	4.70	5005	4600	acid	none	
8b	4.90	7553	7000	acid	none	
8c	7.80	alk.	40	neut. to alk.	medium	
16	4.83	1911	480	acid	none	poor
16a	4.95	4823	3980	str. acid	none	
19	4.83	2366	1180	acid	none	none
19a	4.83	6370	6460	acid	none	
20	4.83	....	920	sl. acid	none	none
20a	4.83	....	4760	acid	none	
23	4.83	....	720	acid	none	none
23a	4.80	....	3960	acid	none	
3	4.45	5915	4240	sl. acid	none	none
3a	4.60	9646	11040	med. acid	none	
17	4.28	4550	2000	acid	none	none
17a	4.75	6734	8080	acid	none	

\* Samples no. 1-19 are from Pendleton County and no. 20-23 from Harrison County. Sweet clover is a very important crop in these counties. It is grown for pasturage, hay and for seed production. The soil, largely of limestone origin, is a brownish yellow to yellow heavy silt loam to clay-loam surface, and a heavy yellow clay loam under soil. The limestone present in the soils is for the most part fragments from the original rock formation and does not represent material added in liming.

† The letter "a" indicates corresponding subsurface sample, 6-18 inches.

‡ Surface, 0-6 inches; a, 6-12 inches; b, 12-18 inches; c, 18-24 inches.

§ Sl. = slightly, v. = very, str. = strongly.

The hydrogen-ion concentration of the sweet clover study samples were determined in January, 1923. The determinations were entirely by the colorimetric method. Clark and Lubs methyl red, brom-cresol purple, brom-thymol blue, and phenol red were the indicators used. The Clark and Lubs standard buffer solutions were also used with the exception of those in the lower range where the acid potassium phthalate was replaced by Sorensen's HCl-citrate solutions. The standards as made up covered the range in steps of about pH 0.3. It was necessary to adjust the reaction of the indicator solutions with small amounts of weak acid or alkali to make the colorimetric readings correspond with the electrometric as tested on the experiment field soils. The colorimetric extracts were made by shaking in a test tube 10 gm. of soil and 15 cc. of water at intervals for 30 minutes, then throwing on filter and pouring back till clear. The reported readings are averages of duplicate readings agreeing within pH 0.10. The pH values as well as the results of the other work are reported in table 3.

These results have not been presented as a basis for any conclusions. The close correlation between limestone treatment or presence of limestone material in soil and hydrogen-ion concentration, however, is to be noted; the reaction of soils treated with limestone or containing natural limestone fragments usually ranged from pH 6.5 to pH 7.5, the untreated acid soils ranged from pH 4 to pH 5.

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## THE DESTRUCTION OF PENTOSANS BY MOLDS AND OTHER MICROORGANISMS<sup>1</sup>

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Wisconsin Agricultural Experiment Station

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The pentoses are five-carbon-atom sugars found widely distributed in nature, chiefly as polysaccharides of high molecular weight which are known as pentosans. Next to cellulose they are the most widely distributed constituent of plant life comprising in some cases as much as 25 per cent of the weight of the plant. A study of the decomposition of these substances in nature by Fred, *et al* (3, 4) indicates that there are present everywhere microorganisms which are able to break down the pentoses and related compounds.

Among the numerous microorganisms involved in the decay of organic matter, none possess a wider range of fermentation power than the molds. They ferment carbohydrates, proteins, fats, alcohols, organic acids, etc., but the destruction of the pentoses and pentose-yielding substances by these forms of life has been investigated to only a limited extent. The rapidity with which pentosan-containing materials are broken down in nature suggests the possibility that fungi play an important rôle in their destruction.

The value of pentose sugars as sources of carbon for fungi has been presented in a recent publication by the authors (8). Among the twenty-five species of molds studied, sixteen were found to ferment the pentoses with rapidity. Most of the remaining nine cultures grew slowly and three or four produced only a few mycelian threads. The best fermenters were found among the *aspergilli* and *penicillia* species although a number of molds of these types attacked the pentoses but slowly. The *Mucors*, *Rhizopus nigricans*, and *Cunninghamella* were also found to be slow fermenters. Four or five days sufficed for the complete destruction of 4 per cent solutions of xylose and arabinose with the most active forms. Compared with glucose, the two pentoses were fermented somewhat less rapidly but the difference was slight. Carbon dioxide and mycelium represented the major portion of the sugars consumed. In 10 per cent concentrations of xylose a considerable quantity of acid was developed and good qualitative tests for citric acid were obtained.

In 1915 Hawkins (5) found that the percentage of furfural-yielding material in the decayed part of an apple inoculated with *Glomerella cingulata* was considerably less than in the sound portion. He also found that xylan and araban are not as readily available for the fungus as the pentose sugars themselves. Tochinali (9) found that *Fusarium lini* can utilize araban. In view of the fact that so little is known concerning the destruction of pentosans by the more common molds, an investigation was undertaken along this line.

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<sup>2</sup> Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

## METHODS

The molds and nutrients used in this work were the same as those reported in a previous publication (8). From the twenty-five species there studied, eight of the most vigorous pentose fermenters were selected for this investigation. The nutrient solution had the following composition:

	cc.
M ammonium nitrate.....	250
M monobasic potassium phosphate.....	100
M magnesium sulfate.....	40
M ferric chloride.....	2
Distilled water.....	608

The individual salts were made up in quantities sufficient to cover a series of experiments. Each stock solution was kept in a separate flask and was combined with the others as needed.

TABLE 1  
*The effect of salts on the determination of pentosans in corn stover*

TREATMENT OF STOVER	PENTOSAN CONTENT
	<i>per cent</i>
Water.....	19.9
25 cc. of salt solution.....	14.6
25 cc. of salt solution minus $\text{NH}_4\text{NO}_3$ .....	20.4
25 cc. of salt solution minus $\text{MgSO}_4$ .....	13.7
25 cc. of salt solution previously distilled with $\text{MgO}$ to drive over the $\text{NH}_3$ .....	12.8
25 cc. of salt solution with $\text{NaNO}_3$ replacing $\text{NH}_4\text{NO}_3$ .....	13.6
25 cc. of salt solution with $\text{NH}_4\text{Cl}$ replacing $\text{NH}_4\text{NO}_3$ .....	20.6
25 cc. of salt solution washed with water on asbestos.....	19.5
25 cc. of salt solution washed with water on asbestos.....	20.3

## DESTRUCTION OF PENTOSANS OF CORN FORAGE

Field corn at the silage stage of maturity was dried and ground to a fine powder which would pass through a 100-mesh sieve. This material was further dried at  $105^\circ\text{C}$ . and accurately weighed amounts, in the neighborhood of one gram, were placed in 150-cc. Pyrex Erlenmeyer flasks containing 25 cc. of the salt solution, and were sterilized for about an hour at 20 pounds of steam pressure. They were then inoculated with the spores of pure cultures of molds and incubated at  $28^\circ\text{C}$ . The fungi grew well on this medium and in a few days the surface of the culture solutions was covered with well developed mycelia. At various intervals the entire contents of some of the flasks were removed and analyzed for pentosans by the method described by Kröber (7).

It was immediately discovered that the nitrates remaining in the culture solution interfered in the pentosan determination by destroying a large part of the furfural. The results in table 1 show the effect of various salts upon the determination of pentosans. The oxidizing effect of the small amount of  $\text{HNO}_3$

secured from the reaction of the HCl upon the  $\text{NH}_4\text{NO}_3$  is clearly shown by a decrease of 30 per cent in the furfural obtained from a distillation performed under such conditions as compared with a normal acid distillation.  $\text{NaNO}_3$  when replacing  $\text{NH}_4\text{NO}_3$  acts in a similar manner, whereas  $\text{NH}_4\text{Cl}$  in the solution is without effect. In one case the ammonia was first removed by distillation with  $\text{MgO}$  but this procedure is not necessary because ammonia does not interfere in the reaction. However, it was found that the corn stover could be transferred to an asbestos filter and the salts removed by washing with distilled water. The residue and the asbestos could then be put in a distilling flask and the pentosans determined in the usual manner. Under these conditions there was practically no loss of furfural-yielding material.

Table 2 gives the percentage of pentosans remaining in the cultures at various intervals. The figures in the last column show that *Aspergillus fumigatus* destroys the pentosans in the corn forage to a greater extent than any of the other molds studied; over 50 per cent disappeared in 142 days. When corn forage was kept in a silo and molds did not develop it was found that about 25-30 per cent of the pentosans were destroyed. The molds were, therefore, about twice as active in the destruction of pentosans as the microorganisms contained

TABLE 2  
Destruction of the pentosans of corn forage by molds

CULTURE NUMBER	MOLD	PENTOSANS REMAINING AFTER						AMOUNT DESTROYED
		0 days	15 days	39 days	46 days	63 days	142 days	
		per cent	per cent	per cent	per cent	per cent	per cent	
15	<i>Aspergillus flavus</i>	20.0	17.1	14.4	....	13.4	11.8	40.1
16	<i>Aspergillus fumigatus</i>	20.0	16.2	15.3	....	10.6	9.4	53.0
5	<i>Aspergillus niger</i>	20.0	15.1	15.4	14.2	....	11.1	44.5
24	<i>Aspergillus oryzae</i>	20.0	....	....	....	....	12.4	38.0
12	<i>Aspergillus repens</i>	20.0	....	....	....	....	13.8	31.0
6	<i>Penicillium glaucum</i>	20.0	17.8	15.8	....	15.0	13.4	33.0
9	<i>Cunningham. sp.</i>	20.0	....	....	....	....	11.5	42.5
8	<i>Rhizopus nigricans</i>	20.0	....	....	....	....	14.1	29.5

in silage. The cultures grew well at first because a large amount of readily available carbohydrate other than pentosans and cellulose is found in corn forage. A larger amount of the pentosans are destroyed during the first few weeks than during the latter part of the fermentation period.

#### DESTRUCTION OF THE PENTOSANS OF RYE STRAW

The rye straw cultures were prepared in the same manner as those grown on corn forage and inoculated with the same molds. Similar cultures were also set up and inoculated with 1 cc. of a soil suspension, and others with 1 cc. of a suspension of cow feces. The organisms did not grow as well as on corn stover, due probably to the lack of readily available carbohydrate such as sugars or



starch. After a month's incubation a light mycelial growth had developed upon the surface of the mold cultures, while the surface of the cultures inoculated with soil suspension and cow feces became covered with a green mold growth. At various intervals the entire contents of some of the flasks were transferred to an asbestos filter, washed with distilled water to remove nitrates, and the pentosans determined in the usual manner.

The last column in table 3 indicates that 33–38 per cent of the total pentosans of the rye straw has been utilized by the fungi in 300 days. It is of interest to note that the pentosans in corn forage are destroyed much more rapidly than the pentosans of the rye straw. A comparison of the soil and cow feces sus-

TABLE 3  
*Destruction of pentosans of rye straw by molds*

CULTURE NUMBER	MOLD	PENTOSANS REMAINING AFTER					AMOUNT DESTROYED
		0 days	29 days	103 days	175 days	300 days	
		per cent	per cent	per cent	per cent	per cent	per cent
15	<i>Aspergillus flavus</i>	26.2	24.3	22.5	21.9	16.2	38.1
16	<i>Aspergillus fumigatus</i>	26.2	21.3	19.3	17.4	17.0	35.1
5	<i>Aspergillus niger</i>	26.2	22.1	20.5	18.4	17.3	33.9
6	<i>Penicillium glaucum</i>	26.2	22.3	20.1	19.9	16.5	36.6
	Soil suspension	26.2	26.4	19.4	18.4	17.0	35.1
	Cow feces suspension	26.2	26.0	20.2	19.6	17.5	33.2

pensions shows that the pure mold cultures can destroy the pentosans as rapidly as can a mixed culture of molds and bacteria. These results prove that the fungi can utilize the pentosans of corn forage and rye straw but not nearly so well as the free pentoses discussed in a previous paper (8) where a complete destruction was secured within a week.

#### DESTRUCTION OF THE PENTOSANS OF WOOD BY MOLDS

In these experiments 1 gram of the finely ground alder, poplar, or birch wood was introduced into 150-cc. Erlenmeyer flasks with 25 cc. of the nutrient salt solution. The ammonium nitrate was replaced by an equivalent amount of ammonium acetate. The flasks were sterilized and inoculated as usual and incubated at 28°C. The cultures would not grow so 1 cc. of a 10 per cent sucrose solution was added to each flask but the molds would not grow even under these conditions. A few of the cultures were analyzed afterwards but the total pentosans had not decreased to any appreciable extent. Evidently some substance was extracted from the woods which inhibited the growth of the molds.

#### DESTRUCTION OF PENTOSANS IN SOIL

In studying the activity of molds in the destruction of the pentosans of various woods it was found that the molds would not grow on a liquid synthetic

medium containing these woods. It was therefore deemed advisable to repeat the experiment duplicating as closely as possible conditions existing in nature.

About a ton of virgin silt loam soil was obtained from Rusk County, Wisconsin. Samples of the brush and young wood growth were taken from the sample place where this virgin soil was obtained. This brush consisted of both green and dry poplar, birch, and tag alder. The soil was sifted through a screen to remove all roots, moss, sticks, etc., and equal weights of soil were placed in 4-gallon jars. The moss was weighed and the weight of moss corresponding to the weight of soil contained in each jar was computed. This gave 300 gms. of moss to be added to each jar to give the soil the same content of moss as would be found under field conditions.

The wood was cut up into pieces 3-6 inches long and  $\frac{1}{8}$ -2 inches in diameter. These pieces were divided into two groups according to thickness. Those under three-fourths of an inch in diameter were designated as "fine" wood, and those above this size were called "coarse" wood. Various weights of these woods were mixed with the different soils. The moisture content was brought up to 20 per cent and maintained at this percentage during the course of the experiment. All the jars were kept in the green house at approximately 30°C.

About two months after the wood had been placed in the soil, oats and red clover were planted in each jar, and two months after planting both top and root of the crops were removed from the jars. The soil was sifted and the residue moss and wood, after the removal of all adhering particles of soil, were dried and weighed. The moss and wood were ground separately to a fine powder and their pentosan content determined in the usual manner. This data is given in table 4.

Only a small portion of the moss could be recovered from the soil and in some cases it had disappeared entirely. It was either completely destroyed or disintegrated to such an extent that it passed through the sieve and so remained with the soil. This probably accounts to a large extent for the increase in pentosans in the soil at the end of the experiment.

Approximately one half of the wood added to the soil was destroyed. The greatest destruction took place in the case of poplar. As would be expected the loss was greater for the fine wood than for the coarse. More than half, 44-67 per cent, of the total pentosans contained in the wood and moss were destroyed. The average loss for the 8 jars was 58.1 per cent.

In table 5 is given a comparison between the destruction of wood and the destruction of one of the wood constituents, the pentosans. An average of over 61 per cent of the poplar wood was destroyed during the course of the experiment; over 50 per cent of the birch wood and about 35 per cent of the alder wood. The pentosans in the various woods were destroyed to the extent of 69 per cent for poplar, 60 per cent for birch, and 47 per cent for alder. It is especially noticeable that in every case the pentosans have disappeared more rapidly than the cellulose, lignin, or other woody constituents, proving that the pentosan complex is more susceptible to the natural processes of decay than the other constituents of the wood.

TABLE 4  
*The destruction of pentosans by the microorganisms of the soil*

MATERIAL	BEFORE PLANTING				AFTER CROPPING				LOSS OF PENTOSANS	
	Weight		Pentosans	gm.	Weight		Pentosans	gm.	per cent	
	gm.	per cent	gm.		gm.	per cent	gm.			
<i>Jar 5:</i>										
Soil.....	11,622	0.102	11.85	11,622	0.110	12.78				
Moss.....	300	9.20	27.60	44	6.31	2.78				
Coarse alder.....	225	22.34	50.27	124	18.07	21.41				
Total.....			88.72			36.97	52.75	58.8		
<i>Jar 6:</i>										
Soil.....	11,622	0.102	11.85	11,622	0.109	12.67				
Moss.....	300	9.20	27.60	26	5.59	1.45				
Coarse alder.....	225	22.34	50.27	148	19.56	28.95				
Total.....			89.72			42.07	46.65	52.0		
<i>Jar 9:</i>										
Soil.....	11,622	0.102	11.85	11,622	0.110	12.78				
Moss.....	300	9.20	27.60	None	.....	.....				
Fine alder.....	450	22.34	100.54	290	16.68	48.37				
Total.....			139.99			61.15	78.84	56.3		
<i>Jar 10:</i>										
Soil.....	11,622	0.102	11.85	11,622	0.123	14.30				
Moss.....	300	9.20	27.60	23	2.52	0.58				
Fine alder.....	450	22.34	100.54	326	19.40	63.24				
Total.....			139.99			78.12	61.87	44.2		
<i>Jar 25:</i>										
Soil.....	11,622	0.102	11.85	11,622	0.130	15.11				
Moss.....	300	9.20	27.60	28	5.68	1.59				
Fine poplar.....	450	21.80	98.10	160	17.28	27.65				
Total.....			137.55			44.35	93.20	67.8		
<i>Jar 26:</i>										
Soil.....	11,622	0.102	11.85	11,622	0.119	13.83				
Moss.....	300	9.20	27.60	19	5.18	0.98				
Fine poplar.....	450	21.80	98.10	190	16.70	32.73				
Total.....			137.55			47.54	90.01	65.4		

TABLE 4—Continued

MATERIAL	BEFORE PLANTING			AFTER CROPPING			LOSS OF PENTOSANS	
	Weight		Pentosans	Weight		Pentosans		
	gm.	per cent	gm.	gm.	per cent	gm.	gm.	per cent
<i>Jar 17:</i>								
Soil.....	11,622	0.102	11.85	11,622	0.138	16.04		
Moss.....	300	9.20	27.60					
Fine birch.....	450	24.60	110.70	218	23.34	50.88		
Total.....			150.15			66.92	83.23	55.4
<i>Jar 23:</i>								
Soil.....	11,622	0.102	11.85	11,622	0.139	16.15		
Moss.....	300	9.20	27.60					
Fine birch.....	450	24.60	110.70	212	17.16	36.38		
Total.....			150.15			52.53	97.62	65.0

TABLE 5  
Comparative destruction of wood and pentosans

WOOD	WOOD DESTROYED		PENTOSANS DESTROYED	
	gm.	per cent	gm.	per cent
Coarse alder.....	101	44.8	28.9	57.4
Coarse alder.....	77	34.2	21.3	42.4
Fine alder.....	160	35.6	52.2	51.9
Fine alder.....	124	27.6	37.3	37.1
Fine birch.....	232	51.5	59.8	54.0
Fine birch.....	238	52.9	74.3	67.1
Fine poplar.....	290	64.4	70.5	71.8
Fine poplar.....	260	57.8	65.4	66.6

## PENTOSAN CONTENT OF MOLDS GROWN ON VARIOUS MEDIA

It has long been known that the mycelium of most molds yields furfural when distilled with hydrochloric acid showing the presence of a pentosan complex. Wichers and Tollens (10) in 1910 found that the mycelium of fifteen species of xylophagous wood-destroying fungi contained 2.5–6.7 per cent of pentosans. In 1911 Dox and Neidig (2) found 0.86–1.17 per cent pentosans in *Penicillium glaucum* and *Aspergillus niger*. They found no purins present. Nucleoproteins, therefore, could not be regarded as the source of the pentoses. They concluded that pentosans are normal constituents of the cell structure of lower fungi and are formed independent of the presence of pentose complexes in the culture media. Ishida and Tollens (6) in 1911 found 2–5 per cent of pentosans in the fungi and 1–2 per cent methyl pentoses. In 1901 Bendix obtained a pentose from tuberculosis bacilli by boiling the dried bacilli with 5 per

cent HCl. The pentose was found to be contained in the nucleo proteins of the bacilli. A mixture of fecal bacteria cultivated in urine and the bacilli of diphtheria both showed the pentose reactions.

Culture solutions were made containing 50 cc. of 4 per cent sucrose and 50 cc. of the nutrient salt solution. These were sterilized for an hour under 20 pounds of steam pressure and 10 of each inoculated with the spores of the various fungi. All of the cultures grew well and produced thick pads, and at the end of seven days a test showed that all the sugar had disappeared. One-half of the cultures were then removed, filtered, and the mold growth thoroughly washed with distilled water. The material was then dried, ground to a fine powder, dried

TABLE 6  
*Pentosan content of the mold growth*

CULTURE NUMBER	MOLD	SUGAR	AGE	DRY WEIGHT AT 105°C.		PENTOSAN	
				days	gm.	mgm.	per cent
15	<i>Aspergillus flavus</i> .....	Sucrose	7	3.010	26.1	0.84	
		Sucrose	30	1.784	15.7	0.85	
		Xylose	10	2.741	36.3	1.32	
16	<i>Aspergillus fumigatus</i> .....	Sucrose	7	3.105	27.5	0.88	
		Sucrose	30	2.154	16.8	0.78	
		Xylose	10	2.621	29.2	0.98	
5	<i>Aspergillus niger</i> .....	Sucrose	7	2.855	21.6	0.76	
		Sucrose	30	2.783	19.4	0.68	
		Xylose	10	1.624	17.7	1.09	
24	<i>Aspergillus oryzae</i> .....	Sucrose	7	2.872	23.8	0.83	
		Sucrose	30	1.241	10.8	0.87	
6	<i>Penicillium glaucum</i> .....	Sucrose	7	2.680	26.8	1.00	
		Sucrose	7	2.678	30.8	1.15	
		Sucrose	30	1.790	21.1	1.18	
		Sucrose	30	2.000	26.0	1.30	
		Xylose	10	2.556	36.6	1.43	

to constant weight at 105°C. and analyzed for pentosans. When the remaining half of the sucrose cultures were a month old they were treated in a similar manner. These cultures appeared dead from the lack of carbohydrate and had apparently decreased in weight.

At the same time an experiment was undertaken to determine whether the organism could deposit more pentosan within its mycelium if grown on a nutrient medium containing xylose as the only source of carbon. Therefore five flasks containing 100 cc. of about 2 per cent crude xylose for each mold was set up in the same manner as was done with the sucrose cultures. After 10 days of incubation a sugar test showed that all the xylose had been assimilated

and the cultures were then filtered and thoroughly washed with distilled water. The combined filtrate did not give any test for reducing sugars. The mold growths were treated as described above. The data are given in table 6.

Apparently pentosans are deposited within the mycelium of the fungi independent of any pentose complex in the culture media. If the fungi are grown on a pentose as a source of carbon a larger amount of furfural-yielding material is deposited within the cells of the mycelium. After all the carbohydrate in the medium is assimilated, the fungus apparently metabolizes some of the pentosan complex which is found in its mycelium as there is a distinct decrease in the amount of pentosans in the mold after it has existed for several weeks on a carbohydrate free medium.

#### SUMMARY

In studying the activities of molds and other microorganisms in the destruction of pentosans it was found that the molds could destroy about 50 per cent of the pentosans of corn forage within 100 days; about 35 per cent of the pentosans of rye straw in 300 days. These organisms would not grow on a synthetic liquid medium with wood as a source of carbon nor did the addition of sucrose result in any growth.

When placed in soil on which a crop was grown, the pentosans of wood were rapidly destroyed. In six months about 60 per cent of the total pentosan content of the wood was destroyed. A larger percentage of pentosans was destroyed than of the wood itself, showing that pentosans are more readily attacked than the cellulose, lignin and other constituents of the wood.

It was found that pentosans are present as a natural cell constituent of the common fungi and are deposited in the mycelial growth to an extent of about 1 per cent, even in the absence of any pentose material in the culture medium. This mycelial pentosan complex can later serve as a source of carbohydrate for the organism. When grown on a medium containing xylose the fungi deposit more pentosans in their cells than when grown on sucrose.

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## MICRO-SAMPLING FOR THE DETERMINATION OF DISSOLVED OXYGEN<sup>1</sup>

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The problem of aeration of the substratum as affecting the development of plants in artificial cultures has been the subject of investigation in this laboratory during the past two years (1, 2, 4). Since it has been found desirable to study the fluctuation of dissolved oxygen in the substratum under the various conditions of these studies, the development of a method of sampling the comparatively small quantities of liquids available has been the subject of special study.

The Mikro-Winkler method suggested by Lund (3) was used in this work. It should be noted that the usual recommendations for sampling emphasize the necessity of protecting the sample to be tested from contact with the atmosphere or from the entrainment of bubbles of air within the sampling device. For the purpose to which it is commonly put, however, as in the case of studying the content of oxygen in large bodies of water, rivers, etc., the size of the sample taken is greatly in excess of that possible under the present conditions. For this reason the following procedure has been developed for use in sampling from such small sources as from one- or two-liter culture vessels with minimum exposure of the sample to the air while sampling and during its subsequent treatment.

In figure 1, the apparatus is shown at *A* ready to draw the sample. The bottle may hold any amount desired, usually about 25-30 cc. The inlet tube *a* is dipped into the solution and suction is applied at the tube *d* to which, for convenience, a piece of rubber tubing may be attached. After filling the bottle, care should be taken to draw out any air bubbles as quickly as possible. The flow is then allowed to continue through the system, the rubber tube acting as the long arm of a siphon, until the volume of the bottle has passed through or until the solution which first filled the bottle is entirely replaced with fresh solution that has not been exposed to the air. After this the pinch cock *C* is closed with the system completely filled. The sample is then ready to be treated with the reagents.

The manner in which this is done without exposing the sample to the air is shown in figure 1 at *B*. The part *a* of the inlet tube is removed and the

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portion *b* remains in the bottle, being permanently fixed in the stopper. The outlet tube *d* is also removed at the same time, care being taken to keep the bottle in an upright position during the process so that no air may enter through the opening in the stopper. Then by inserting into the opening in the stopper at *r* a 1-cc. pipette with graduation intervals of 0.01 cc. the necessary reagents may be readily added, the flow from the pipette being controlled by the pinch cock at *C*.

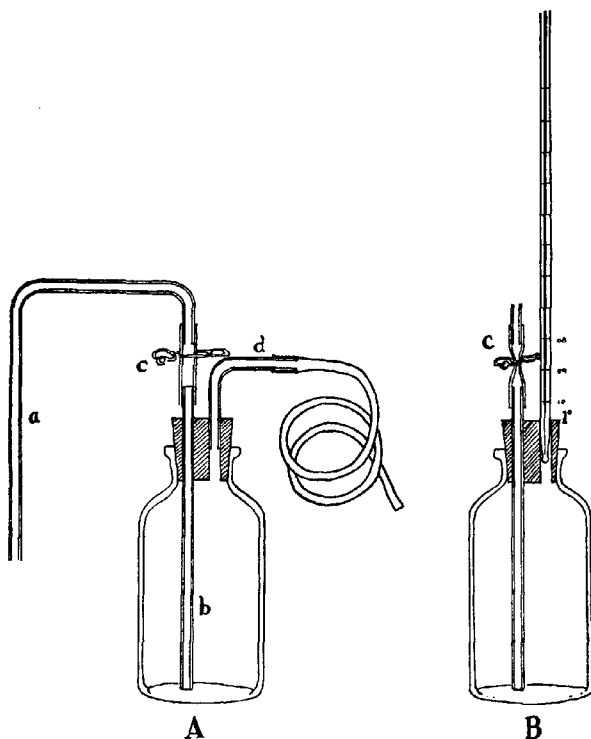


FIG. 1. DIAGRAM OF SAMPLING APPARATUS FOR OXYGEN DETERMINATIONS

After the addition of the reagents and their proper mixing with the sample by rotating the bottle the stopper may be removed. It is important to keep the pinch cock *C* closed while removing the stopper so that the solution displaced by the addition of the reagents and still remaining in the tube may not drop back into the treated sample. After the removal of the stopper from the bottle the titration may be carried out in the usual manner.

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